EFFECTS OF LOW TEMPERATURE ON THE PHOTOSYNTHESIS AND PRODUCTIVITY OF RICE PLANTS

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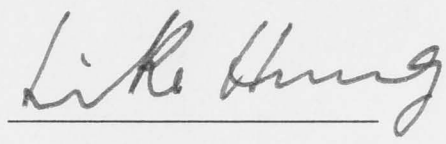
Statement

The experiments reported in this thesis are my own work, done under the supervision of Professor C. B. Osmond, Drs. S. C. Wong, and I. Terashima, in the Plant Environmental Biology Research Group, Research School of Biological Sciences, ANU. Publications arising from this work, and from other research collaborations, were largely prepared by my colleagues and are listed below. I used these papers to guide the preparation of the first draft of Chapters 3 and 4 of this thesis, which were prepared by myself. I am indebted to Drs C. B. Osmond and S. C. Wong for the substantial assistance given in revision of the final draft of the thesis. Without this help my written English would have been much less clear. Moreover, specific contributions by the others will be referred to in the acknowledgments.



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ABSTRACT

Yield of second crop rice (*Oryza sativa L.*) in South-East China is susceptible to a sudden drop in air temperature below 23 °C for 3 days or more during heading/flowering and grain filling. This particular weather condition which affects rice plants is known as "Cold Dew Wind" (CDW). This thesis explores the hypothesis that the effects of the chilling injury on yield may be mediated its effect on concurrent photosynthesis.

The results in this thesis are concerned with three main research directions, which can be summarized as follows:

(1)Primary processes of photosynthesis in rice indicated by chlorophyll flourescence and quantum yield of O_2 evolution at CO_2 saturation, were impaired by bright light at low temperature. Attached and detached leaves of rice cultivars differ in their sensitivity to the interaction between high light and low temperature; low temperature exaggerated photoinhibition. Recovery from photoinhibition occurred in the dark at warm temperature (25 °C) conditions, but not in low temperature conditions (10 °C). There was no difference in the subsequent response of photosynthesis in leaves in different cultivars when exposed to low temperature in the dark; low temperature pretreatment in the dark and then exposure to high light did not exaggerate photoinhibition.

(2) Canopy photosynthesis was reduced by chilling stress, particularly under simulated Dry Cold Dew Wind (DCDW) conditions in controlled environment growth chambers.

There was a correlation between photosynthesis and carbohydrate accumulation in leaves of different chilling sensitive rice cultivars. Midday depression of photosynthesis was caused by low root temperature, and varied in different chilling sensitive cultivars, and might be due to increase in soluble sugars accumulation in the leaves. There was little correlation between photosynthetic CO_2 assimilation and changes in primary processes of photosynthesis indicated by 77K flourescence; the extent of low temperature photoinhibition in the canopy under simulated DCDW environment conditions was dependent on leaf orientation.

(3) Yield reduction in different rice cultivars under the simulated CDW conditions was due to: (a) a reduction of photosynthesis probably caused by the accumulation of soluble sugars in the leaves; (b) a direct effect of low temperature on grain sterility. In relatively chilling tolerant cultivars of rice, part ofthe reduction in grain yield was due to lower photosynthetic assimilation and the limitation in photosynthate translocation. However, in chilling sensitive cultivars, there were large effects on photosynthesis, but grain sterility was also very important factor in reducing yield.

The results suggest that: (1) measurements of chlorophyll fluorescence as a rapid method for screening chilling tolerant cultivars in rice plants may need further investigation to substantiate its usefulness; (2) measurements of the accumulation of soluble sugars in the leaves should be considered for screening of chilling tolerant rice cultivars.

Abbreviations & Symbols

A - CO_2 assimilation

ADP - Adenosine 5′-diphosphate

ANU - Australian National University

ATP - Adenosine 5′-triphosphate

CDW - Cold dew wind

chl.*a* - Chlorophyll a

chl.*b* - Chlorophyll b

DCDW - Dry cold dew wind

DMF - N,N-dimethylformamide

E - Transpiration rate

g - Stomatal conductance

F - Fluorescence

F_m - Maximum fluorescence, Q reduced

F_o - The initial, instantaneous level of fluorescence, Q oxidized

F_p - Maximum fluorescence, Q reduced

F_R - The maximum rate of rise of the fluorescence induction curve

F_t - Final fluorescence

F_v - Variable fluorescence, $(F_m - F_o)$

IRGA - Infra red gas analysis

LHCP - Light-harvesting chlorophyll protein

NADPH - Nicotinamide-adenine dinucleotide phosphate (reduced)

PFD - Photon flux area density

PSI - Photosystem I

PSII - Photosystem II

Q_{10} - Ratio of the rate at one temperature to that at a

temperature 10 °C lower

TNSC - Total non-structural carbohydrate

WCDW - Wet cold dew wind

CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

1. 1. BACKGROUND TO EFFECTS OF LOW TEMPERATURES ON RICE YIELD IN SOUTH-EAST CHINA

A farmer's proverb in South China, which can be translated as **"Standing grain (rice) is afraid of Cold Dew Wind"**, is one of the best indicators of an ancient problem in rice cultivation in this region. Low temperature frequently reduces the yield of the second crop of rice in its late stages in South-East China. Guangdong Province, located in the South-East of China, extends from the South-Ridge of the Five Ridges to the Pacific Ocean (the South China Sea). The paddy fields of this province extend across a vast area of the tropics and subtropics. It is one of major rice producing areas of the world, and is the second largest rice growing area in China. Approximately 24,000 km^2 of Guangdong Province (11% of total area) is cultivated with rice. It is also the second largest yielding region. In South-East China the weather patterns responsible for the low temperature-induced reduction in the yield of second-crop rice are known as "Cold Dew Winds" (CDW).

"Cold Dew Winds" are weather phenomena which occur when the East Asia cold high pressure front moves to the south. The South-Eastern Monsoon is established, and a cold air current invades South China. Normally, CDW occur during the turn of the Autumn and Winter seasons, and this is identified as the "Cold Dew" season, one of the Twenty-four Solar Terms of the Chinese Agricultural Calendar.

Thus, the severity and duration of the CDW can lead to a disaster in the rice production of South-East China. Hundreds of thousands of tonnes of rice yield can be lost in each province. In the years of most serious drop in production planting of the second season rice was postponed because the first crop was delayed by low temperature in the early spring season ("Dao Chun Han", unusual early spring season chilling). The first crop rice harvest was postponed, increasing the risk of low temperature damage to the second season crop later in the season.

Some examples of CDW conditions can be taken from unpublished data collected by Lin, *et al.* (1986). In 1976, there were two relatively strong CDW events which occurred at the end of September and mid-October. This was followed by a strong cold air current in mid-November. The mean grain yield per unit area was reduced by 0.37 tonnes ha^{-1} in Guangdong Province. The mean grain yield was about 4 tonnes ha^{-1} for the second crop. During these periods the temperatures dropped 4-7 °C below those in normal years. The mean of temperature during these CDW was below 13 °C, and the minimum temperature dropped to 7.8 °C. There was also serious seedling damage under cloudy weather conditions with chilling injury in the first crop for this year. Thus, the harvest of the first crop was deferred, and the whole growth season for the second crop was postponed. Moreover, the #19 Typhoon also invaded Western Guangdong this year, making it one of the worst rice yield years on record.

In 1978, another special year of serious grain yield reduction occurred when the #20 typhoon invaded Guangdong on 8 October, followed by a strong CDW in the middle ten days of October. Temperatures were lower than 20 °C for 5 days in the Guangzhou area. A second CDW event on 28-31 October

which followed the #25 stronger typhoon, and the mean temperatures during this event were 15-16 °C, with a minimum of 9.5 °C. These two low temperatures events seriously damaged the late season rice crop in the whole Province of Guangdong. The total output and the yield per unit area was reduced by 14% compared to the previous year. It was the most serious CDW event since 1951. In both 1976 and 1978, the rice grain yield loss was over 2 million tonnes, valued at about 400 million RMB yuan (approx $200 million).

The most recent CDW occurred in 1981 when the cold air currents arrived on 9 October and 23 October in Guangdong province. In addition, there were typhoons, storms and generally poor climate during the early and middle developmental stages in the second season crop in this year, so that about 1 million tonnes of rice were lost in this province.

Statistics based on the details of the meteorological record in the Guangzhou area from 11 September to 20 October during 1951-1985, show that CDW occurred in 25 of these 35 years, and the probability of occurrence was 71.4% (Anon, 1974). The traditional definition of a CDW event is a period of three days or more in which mean daily air temperature is 23 °C or less. The earliest CDW occurred on 25 September 1957, and the latest on 1 November 1953. The longest total of CDW periods was 9 days in one year, and the longest single CDW period was 8 days. The probability of appearance of CDW before the "Cold Dew" season (normally on 8 October) was 32%. Data have been collected in various locations in Guangdong Province and are shown in Table 1.1. Geographical designations of regions differing in susceptibility to the CDW have been classified and shown in Figure 1. 1.

Two forms of these events have been recognised (Anon, 1974). The "Dry Cold Dew Wind" (DCDW) is characterised by low night temperatures and bright, dry days which are cool with strong winds. The "Wet Cold Dew Wind" (WCDW) is characterised by little variation in day/night temperatures, and by cloudy, dull days with rainfall and strong winds. In the 25 CDW events mentioned above, 14 (56%) were DCDW, and 11 (44%) were WCDW years. Typical DCDW occurred in 1954 and 1955, and typical WCDW occurred in 1970, 1976 and 1978. The tendency for appearance of DCDW and WCDW has been changing. The probability of the occurrence of DCDW has decreased since the 1970'S.

Researchers at the Guangdong Rice Research Institute, Guangdong Academy of Agricultural Sciences, and at the South China Institute of Botany, Academica Sinica, indentified low temperature depression of rice yield due to CDW, as the top priority, physiological limitation to rice production in South China. A collaborative research project, sponsored by the Australian Centre for International Research, was initiated in 1986, to study this problem. The research reported in this thesis is part of this project.

Table 1. 1. Some characteristics of Cold Dew Winds at four location in Guangdong Province (Data from Anon.1974)

Location	Earliest [A]	Latest [B]	Maximum [C]	Minimum [D]	Longest [E]
Guangzhou	25/9 (1957)	1/11 (1953)	9 (1954)	0 (7 yrs)	8 (1954)
Huiyang	23/9 (1967)	5/11 (1964)	12 (1957, 1971)	0 (4 yrs)	12 (1954)
Shaoguan	20/9 (1971)	21/10 (1951, 1953)	22 (1957)	0 (2 yrs)	11 (1968)
Nanxiong	14/9 (1967)	20/10 (1963)	26 (1967)	3 (1954)	18 (1969)

A The earliest CDW record.

B The latest CDW record.

C Maximum of number of total CDW days.

D Minimum of number of total CDW days.

E The longest CDW event of one period.

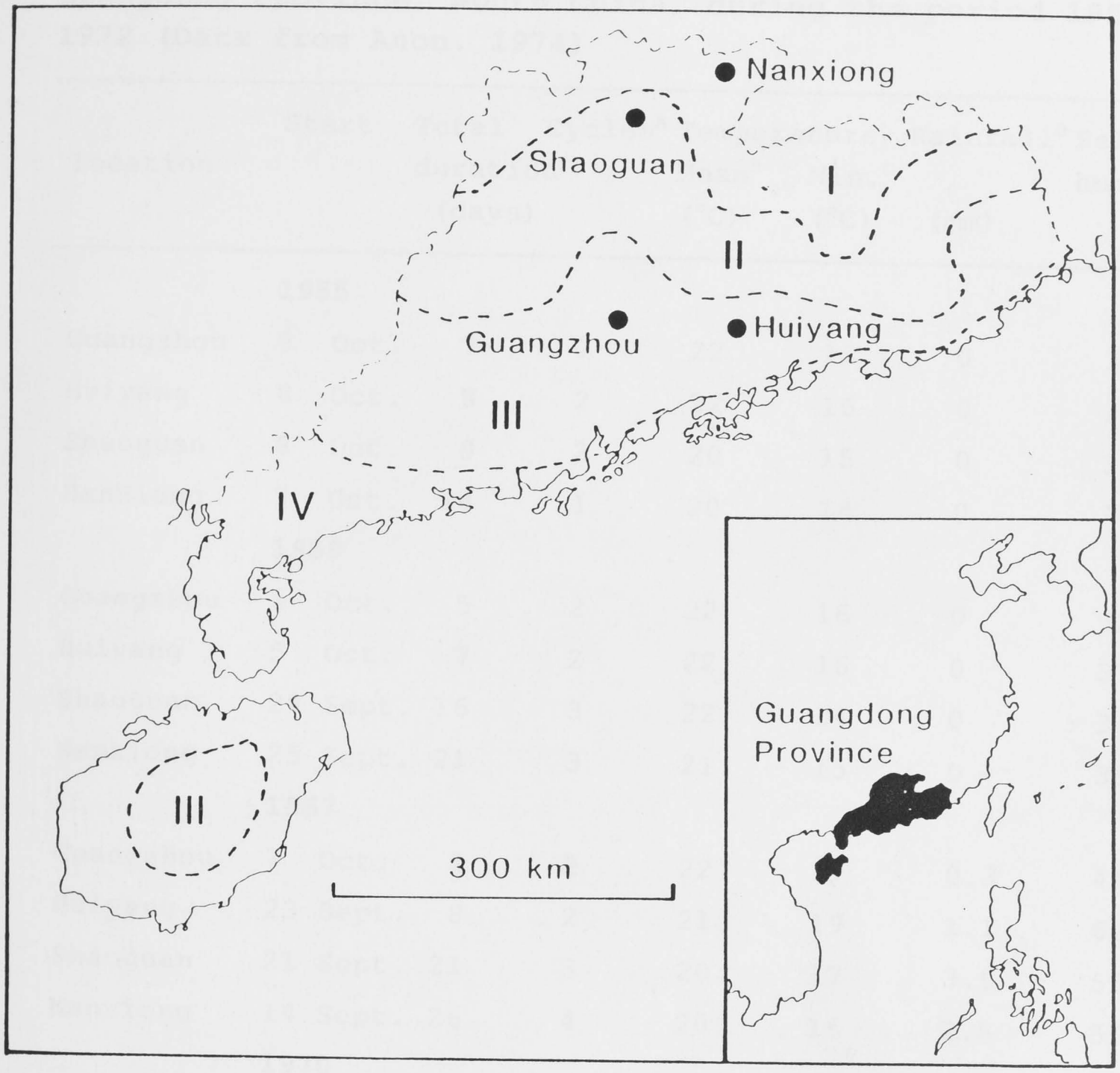

Figure 1. 1. Map of Guangdong Province, South China, showing locations used for the climatic records given in Table 1. 1. Regions correspond to strong (I), medium (II), weak (III) unlikely (IV) susceptibility to Cold Dew Winds climatic events. In the years 1963-1972, the total number of CDW days in each region was: I = 120; II = 75; III = 30 and IV < 30. The commencement of CDW days in region I (20/9) is earlier than II (30/9) or III (10/10). Likewise, the mean duration of CDW days in region I (14-17 d) is greater than in II (8-12 d), III (4-6 d) and IV (< 3 d) (redrawn from Anon, 1974)

Table 1. 2. Climatic characteristics of the four strong Cold Dew Winds events recorded at four locations in Guangdong Province, South China, during the period 1951 - 1972 (Data from Anon. 1974)

Location	Start	Total duration (days)	Cycles[A]	Temperature Mean[B] (°C)	Temperature Min.[C] (°C)	Rainfall[D] (mm)	Relative humidity[E] (%)
	1955						
Guangzhou	8 Oct.	7	2	22	18	0	36
Huiyang	8 Oct.	8	2	22	16	0	38
Shaoguan	8 Oct.	9	2	20	15	0	32
Nanxiong	7 Oct.	14	1	20	14	0	36
	1959						
Guangzhou	5 Oct.	5	2	22	16	0	32
Huiyang	5 Oct.	7	2	22	16	0	37
Shaoguan	28 Sept.	16	3	22	14	0	34
Nanxiong	25 Sept.	21	3	21	15	0	38
	1967						
Guangzhou	7 Oct.	2	2	22	17	0.3	49
Huiyang	23 Sept.	8	2	21	19	1.1	62
Shaoguan	21 Sept.	21	3	20	17	3.9	56
Nanxiong	14 Sept.	26	4	20	16	2.8	55
	1970						
Guangzhou	29 Sept.	3	1	21	18	4.6	62
Huiyang	30 Sept.	3	1	21	18	0	56
Shaoguan	29 Sept.	11	2	20	17	13.1	61
Nanxiong	29 Sept.	13	2	20	16	7.1	63

[A] Cycles defined by 2 or more days with mean daily temperature of 21 °C or less; or 3 or more days with mean daily temperature below 23 °C.

[B] Mean of mean daily temperatures recorded at 0200, 0800, 1400 and 2000 hours.

[C] Mean of daily minimum temperature.

[D] Mean of total daily rainfall.

[E] Mean of relative humidity recorded at 1400 hours.

1. 1. 1. Characteristics of yield reduction in rice due to "Cold Dew Winds"

Agronomists and agrometeorologists have collected many details of low temperature effects on second crop rice, because they consider that the main reason for low grain yield in South-East China is low temperature. In general, the heading-flowering and grain-filling, milky stages are all sensitive to low temperatures, leading to increased percentage of empty grains (sterility) and half-filled grains, with a consequent reduced percentage of filled grains and/or reduced thousand grain weight. World-wide, the most common response is that low temperatures restrict flower development in the caryopsis, leading to sterility and to a high percentage of empty grains. Long-term observations in South China show the most serious reduction in yield is when the CDW occurs during the flowering stage in rice. The most severe damage is found when CDW occur during early flowering, when sterility may be as high as 30-40%. When CDW occur at the end of flowering/early grain-filling stages the effect decreases to 10-15% empty grains, with increased percentage of half-filled grains.

Peterson *et al.* (1974) showed that low temperature 10 - 14 days before heading reduced microsporogenesis. Yu *et al.* (1980) and Hu (1981) indicated that this response to low temperature before or during flowering was an important factor. Laboratory studies showed that low temperature increased the percentage of empty-grains in the rice panicle, and that many factors may lead to these results.

Chinese researchers showed that the pollen grains were more sensitive to low temperature than the ovary and stigma (Shanghai Institute of Plant Physiology, phytotron, 1975). Hu (1981) reported that, although the anthers opened and the pollen was spread, pollen germination and pollen tube growth on the style were impeded at low temperatures.

Low temperature depression of rice yield in South China usually involves other responses. Tu, (1981) analysed the interaction between the grain yield of the late season rice and meteorological phenomena for the 12 years 1970 to 1981. He pointed out, that in the plains area in the centre of Guangdong, 60-70% of the yield reduction was due to CDW which occurred during the grain-filling milky stage, and this increased to over 90% in the mountainous area in the centre and north of Guangdong. He further analysed the meteorological records of 18 years from 1964 to 1981 and found low temperature injury in the late milky stage occurred in 9 of these 18 years and which reduced the grain yield by 67%. Yu *et al.* (1980) have shown that in a simulated chilling treatment (mean temperatures of 16-19 °C for 3 days), there was a reduction in the percentage of filled-grains and the thousand grain weight compared with 20-22 °C temperatures. Effects other than low temperatures were also important. During the DCDW, Tu, (1981) found rice leaves aged prematurely, and grains failed to develop, possibly because of water stress. Yu *et al.* (1980) also observed an abnormal change in leaf structure, involving deformation of bulliform cells, suggesting perhaps that leaves were water stressed.

In my experience, low temperatures can have different effects depending on the time of development that the temperature treatment is given. As reported by Zhang *et al.*

(1984a), the effect of decreasing temperatures to below 18 °C for 3 days at the rice heading-flowering stage was to reduce the ratio of flowering (increased empty grains) and to reduce the percentage of filled-grain. In the same laboratory, He *et al.* used an X-ray technique to trace caryopsis development before, during, and after simulated chilling treatment at the grain-filling, milky stage. When chilling was applied during the early grain-filling milky stage (which occurs 5 days after heading), low temperature increased the percentage of half-filled grains, reduced the rate of grain filling, but did not reduce the thousand grain weight of filled-grain. The rate of grain filling recovered quickly when plants were returned to natural weather conditions.

These studies suggest that supply of photosynthetic products may directly affect the yield of rice plants when exposed to low temperature in the grain-filling stages. This may thus represent a distinctive feature of low temperature, CDW effects on rice yield in South China, compared with other regions. For this reason, the research described in this thesis has concentrated on photosynthetic processes.

1. 1. 2. Physiological aspects of yield reduction in rice at low temperature

Rice physiologists have considered many hypotheses to explain low temperature effects on rice yield. For example, it is well known that Indica-rice, of the types grown in South China, has lower photosynthetic rate at low temperature than Japonica-rice (Tanaka, 1976; Park and Tsunoda, 1983; Evans and Bush, 1985). Kishitani and Tsunoda (1974) showed that a brief, three day treatment at 17 °C day/12 °C night was enough to strongly depress photosynthesis in Indica-rice, with little effect on Japonica-rice. Similar differences are shown in Figure 1. 2. from experiments done by Terashima, and published by Huang *et al.* (1989 b). He *et al.* (1987 a,b) reported that chilling temperatures reduced the flag leaf photosynthetic rate, increased stomatal diffusive conductance, and that chloroplast development was hindered, and PEPase activity was reduced, in the hybrid Indica-rice (cv. Sang Yui-6). Chilling temperatures (under 15 °C) reduced the maximum photosynthetic rate and the quantum yield, but both these parameters recovered to the control level in the dark, and recovered more slowly under high light conditions.

A popular hypothesis to explain temperature responses focuses on the stability of membranes at low temperature which may be related to the extent of unsaturated fatty acids in the membranes (Su and wang, 1983). Zeng *et al.* (1987 a,b) showed that chilling temperatures limited the activity of superoxide dismutase (SOD), so that the protective action

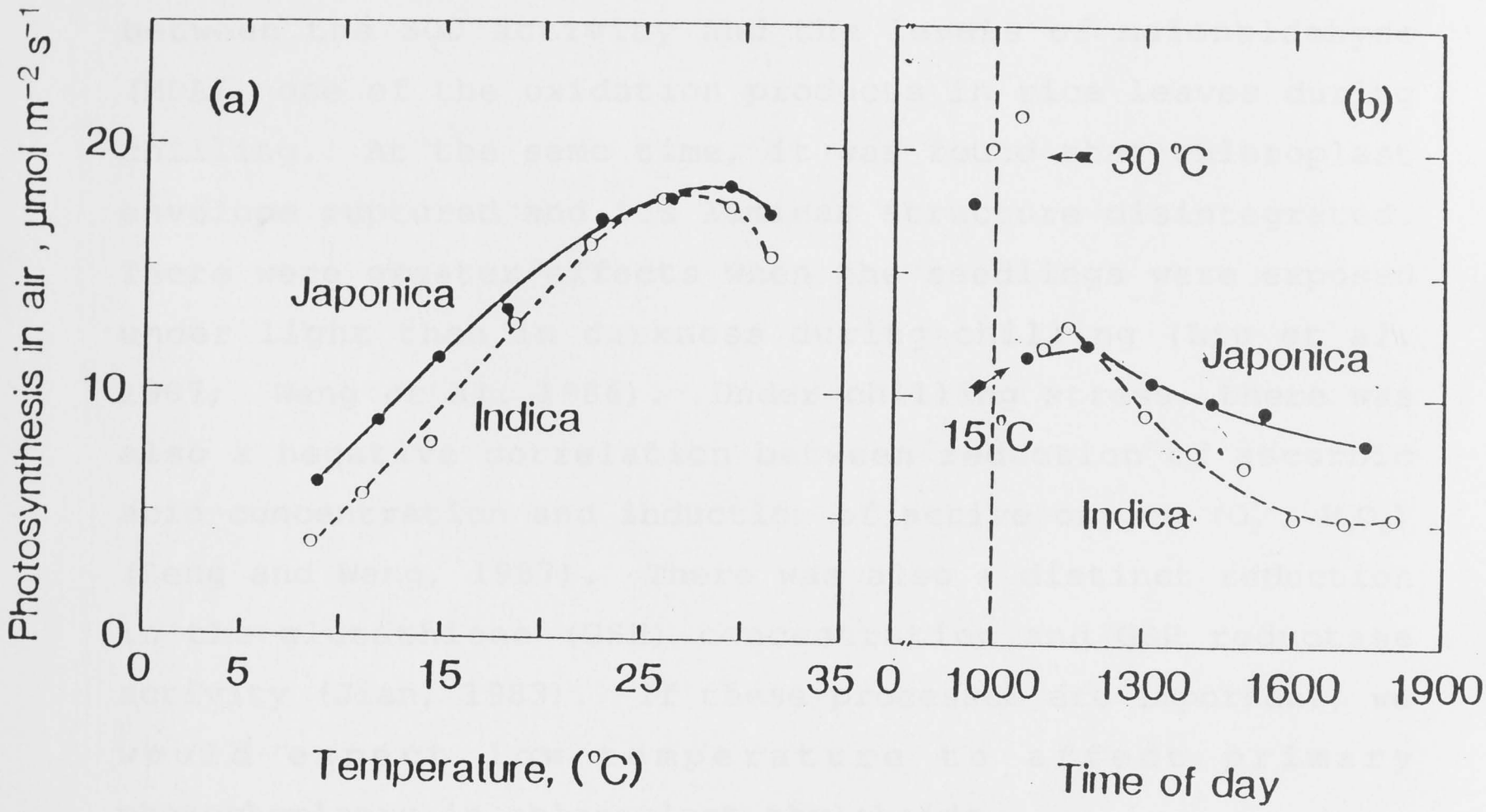

Figure 1. 2. (a) Temperature reponse curves for photosynthesis in air of rice cultivars grown at 30 °C day/20 °C night; (●) Japonica rice (cv. Hungarian-1); (O) Indica rice (cv. IR-8). (b) Time curves of change in photosynthesis in air in rice cultivars transferred from 30 °C to 15 °C at the time indicated; symbols as above. (Data of Terashima in Huang et al 1989b).

of this enzyme on the membrane system was reduced in rice seedlings, and amount of the free-radical *in vivo* increased. Thus membrane lipid oxidation was aggravated, causing damage. Liu *et al.* (1987) found a negative correlation between the SOD activity and the levels of malonaldehyde (MDA), one of the oxidation products in rice leaves during chilling. At the same time, it was found that chloroplast envelope ruptured and its laminar structure disintegrated. There were greater effects when the seedlings were exposed under light than in darkness during chilling (Liu *et al.* 1987; Wang *et al.* 1986). Under chilling stress, there was also a negative correlation between reduction of ascorbic acid concentration and induction of active oxygen (O_2^-, H_2O_2) (Zeng and Wang, 1987). There was also a distinct reduction in the glutathione (GSH) concentration and GSH reductase activity (Jian, 1983). If these processes are important, we would expect low temperature to affect primary photochemistry in chloroplast thylakoids.

Other experiments show that low temperature stress depresses the export of photosynthates from the flag leaf, as well as translocation to other organs. The conversion of soluble ^{14}C-compounds into acid-insoluble compounds is disturbed and the biosynthesis of macromolecular compounds from $Na_2H^{32}PO_4$ are greatly altered in the cold (Fu *et al.* 1983). The translocation and distribution of the photosynthetic products into the roots were especially impeded. Also reduced root activity and reduced translocation of ^{32}P from roots to the panicles at low temperatures has been observed (Wang *et al.* 1980). Because impaired translocation leads to sucrose accumulation in leaves of rice, and inhibition of photosynthesis (Kishitani and Tsunoda, 1974), we need to consider this effect.

1. 2. LOW TEMPERATURE EFFECTS ON PHOTOSYNTHETIC PROCESSES

The effect of low temperature on photosynthesis has been studied extensively, and effects on most of the physiological and biochemical reactions and processes have been described. It is necessary to define the temperature range of relevance in any investigation, and the term 'chilling' is usually used to describe temperatures above freezing and below 20 °C. For the purposes of this thesis chilling refers to effects in the range of 10 - 20 °C. It is clear that chilling reduces the maximum photosynthetic rate (Taylor and Rowley, 1971). There are two major ideas in the literature to explain this reduction in photosynthesis at low temperature.

Firstly, it is thought that chilling directly affects the structure of photosynthetic organs, especially membranes, and as result, reduces biochemical activity of photosynthesis. At least three types of interaction have been studied:

(1)Changes in the fluidity and permeability of the thylakoid membranes (Lyons and Raison, 1970; Wolfe, 1978; Lynch and Thompson, 1982), associated with changes in chloroplast ultrastructure (Kimball and Salisbury, 1973; Levitt, 1980; Sato and Park, 1982; He, 1985) and also changes in chlorophyll concentration (Friend, 1960; Hasselt, 1972) and changes in the ratio of chlorophyll a to chlorophyll b (Roy and Biswas, 1981; Paliombetora *et al.*, 1981).

(2) Chilling temperature has an effect on the primary photochemical reactions in photosynthesis and subsequent electron transport and photophosphorylation (Critchley, 1981; Smillie and Nott, 1979; Smillie *et al*. 1988; Powles 1984; Martin and Ort, 1982; Moll and Steinback, 1986; Peeler and Naylor, 1988; Chow *et al*. 1989).

(3) Effects of chilling on the photosynthetic dark reactions, primarily through the activities of enzymes in the Calvin Cycle (Graham and Patterson, 1982; Weeden and Buchanan, 1983; Nakamoto and Edwards, 1983; Leegood and Furbank, 1986; Labate and Leegood, 1988).

Secondly, chilling has an indirect effect on photosynthesis, due to a dysfunction of some of the other physiological processes effected by the chilling conditions. For example:

(1) Chilling was found to induce water stress (Peoples and Kock, 1978; Long and Woolhouse, 1978; McWilliam *et al*. 1982).

(2) Chilling increases the diffusive conductance of stomata to CO_2 (Crookstoon *et al*. 1974; Charles-Edwards *et al*. 1971; Drake and Salisbury, 1972; Neilson *et al*. 1972; Neilson and Jarvis, 1975).

(3) Chilling has effects on the translocation of photosynthates from leaves, leading to increased accumulation of carbohydrates in leaves (Hillard and West, 1970; McWilliam *et al*. 1982; Chatterton, *et al*. 1972; Geiger, 1969; Hofstra and and Hesketh, 1975; Levitt, 1980; Park and Tsunoda, 1983; Wilson, 1979; He, 1985).

In natural field conditions, however, photosynthesis could be affected by all of the other environmental factors, such as light intensity, water availability, nutrition, CO_2 and O_2 concentration in the atmosphere, which interact with low temperatures. In the last few years, particular attention has been given to the interaction between light and low temperature on photosynthesis. A number of reports have verified that the photosystems are damaged under chilling stress in many plants (Ögren *et al.* 1987; Osmond *et al.*1987).

1. 2. 1. Effects of chilling in the dark on photosynthetic processes

In recent years, there have been many studies of electron transport in thylakoids to understand the interaction between low temperature (chilling stress) and photosynthesis in higher plants. When chloroplasts were isolated from bean leaves after treatment at 0°C in the dark for two days, electron transport activity was reduced. Measurements showed that the site of damage was in the water splitting machinery of photosystem II (PS II), but there was no effect on photosystem I (PS I) (Margulies, 1972). The results of other experiments showed detached leaves of tomato were more damaged at 0°C in darkness than when the whole plant was exposed under 0°C with moderate light intensity. Again, the primary damaged site during chilling in the dark was PS II (Kaniuga *et al.* 1978). Kaniuga and his colleagues considered PS II damage was accompanied by a reduction in Mn^+, increase of the fatty acid concentration and degradation of protein and ATP.

Cucumber is often considered a typical chilling-sensitive plant, and there have been many studies of the effects on photosynthesis (Critchley 1981; Critchley and Smillie, 1981). After comparing the chilling tolerance of cucumber and Indica-rice (cv. IR-8), Terashima concluded that the sites of chilling damage in cucumber thylakoid differ depending on the presence or absence of light (Terashima *et al.* 1989). In the dark, the sites of chilling damage was the water splitting machinery of PS II, confirming Critchley (1981). The coupling factor was the site damaged by chilling with light. In rice plants, both chilling stress in the light or in the dark hardly affected photosynthetic functions of thylakoid, even though chilling in the dark brought about a longer induction period for photosynthetic oxygen evolution in leaf discs. So rice thylakoids are quite resistant to chilling treatments (Terashima *et al.* 1989). These results were confirmed by Smillie *et al.* (1988) using different techniques.

There are other reports, however, which are sceptical of this viewpoint. Isolated chloroplasts separated from the leaf of tomato plants, which had been treated at 1°C for 16 hours in the dark, showed few changes in PS II activity (Martin and Ort, 1982), although Kee *et al.* (1986) subsequently reported extensive PS II damage after longer exposure under these conditions. These authors also confirmed earlier reports which point out that a chilling sensitive plant is more damaged by chilling in bright light than in the dark (Taylor and Rowley, 1971; He *et al.* 1987 a,b). Such observations raise the question of photoinhibition as a factor in low temperature effects on photosynthesis.

1. 2. 2. Effects of chilling in the light on photosynthetic processes

Chilling injury to photosynthesis occurs to a greater extent under high irradiance than in darkness. The site of damage also varies depending on whether the plants are irradiated during chilling (Powles, 1984; Taylor and Craig, 1971; Van Hasselt *et al.* 1980; Terashima *et al.* 1989). Therefore, one might suggest that low temperatures have a direct effect on photosynthesis in chilling-sensitive plants, thus making them particularly susceptible to excessive excitation (Long *et al.* 1983; Powles *et al.* 1983). In chilling-resistant plants, however, chilling temperature as such apparently causes no detectable dysfunctions in photosynthesis, but the synergism between light and chilling temperatures may still inhibit photosynthesis (Öquist *et al.* 1987).

Light-dependent damage to photosynthesis is called photoinhibition, which was defined by Kok (1956) and Kok and Businger, (1956) as a "narcotic action" of high light intensity. This is a very general and useful definition which can be taken as a starting point for understanding many light dependent stresses. Osmond (1981) proposed that photoinhibition will be observed under any circumstances in which the rate of transfer of excitation energy from light-harvesting pigment assemblies to photochemical reaction centres, is in excess of the rate of transfer of excitation energy from the reaction centers to the electron transfer chain. In intact plants, when photoinhibition occurs, changes in chlorophyll fluorescence are observed. Exposure

to excessive light at chilling temperatures results in a decrease in F_v/F_m, and increase in F_o at 77K temperature which indicate effects on primary photochemistry of PS II (Ögren and Öquist, 1984 b). Typically, photoinhibition of photosynthesis also results in an inhibition of the maximum quantum yield of CO_2 uptake, indicating impaired photochemistry (Powles *et al.* 1983; Long *et al.* 1983).

Chlorophyll fluorescence parameters have been used to demonstrate differences in temperature-sensitive plants between species and cultivars (Hetherington *et al.* 1983). However, the potential to apply these methods to distinguish difference in sensitivity to photoinhibition remains to be explored (Smillie *et al.* 1988).

1. 3. THE AIMS OF THE THESIS

The thesis describes part of the research done in the ACIAR project on interaction of low temperature and light as a factor limiting rice yield. It concentrates of measurement on responses in whole plants, but also describes experiments with excised leaves. The overall objective was to find responses which could be used for screening rice cultivars for chilling-tolerance. Three main research questions were studied:

(1) Do leaves of rice cultivars differ in their sensitivity to interaction of light and low temperature, as indicated by chlorophyll fluorescence and the quantum yield of photosynthesis?

(2) Do the above processes indicate damage to photosynthesis under conditions of simulated CDW events in controlled environment? If they do not, are other physiological processes correlated with decreased photosynthesis under these conditions, and can these be used to screen cultivars for low temperature tolerance ?

(3) How useful are these techniques for selecting low temperature tolerance of cultivars under field conditions?

This thesis is organized into four major parts. The first part (Chapter 1 and Chapter 2) is devoted to a review of information on Cold Dew Winds and their effects on the yield of rice, and of research into plant chilling stress, particularly on the rice. It also describes the methods used to study rice in the laboratory and the field, including photosynthetic gas exchange from intact tissues and whole plants, chlorophyll fluorescence from PS II, and carbohydrate accumulation.

The second part (Chapter 3) concentrates on the laboratory experiments primarily concerned with chilling injury in the mature leaves of rice. Cultivar differences in the interaction between low temperatures and light, were measured by chlorophyll fluorescence at 77K and quantum yield of photosynthesis.

The third part (Chapter 4) describes effects of chilling stress on canopy photosynthesis under simulated DCDW conditions experienced in South-East China. Cultivar differences were described, and seem to be reflected in the relationship between carbohydrate accumulation and photosynthetic rate.

The fourth part (Chapter 5) describes similar experiments in which yield parameters were measured, as well as the results of field experiments in which different cultivars were compared under natural conditions in South-East China. Unfortunately, no naturally accuring CDW events have been observed in the field during the course of this thesis. Nevertheless, the discussion shows how these experiments give new understanding of physiological responses of rice to CDW, and suggest new methods for screening chilling tolerance in rice cultivars.

CHAPTER 2. EXPERIMENTAL METHODS

2. 1. INTRODUCTION

As outlined in the aims of this thesis, the experimental techniques used to study rice were chosen with a view to distinguish the responses of cultivars to low temperatures, and at the same time, to obtain some understanding of physiological characteristics of photosynthesis at low temperatures in relationship to yield. Thus, most of the methods have been applied to leaves, both attached and detached. The environmental conditions used in experiments were chosen so that they matched natural conditions as much as possible. Because Cold Dew Winds have been traditionally described in terms of mean daily air temperatures, it was necessary to make more accurate measurements of leaf temperature, as the background to planning laboratory experiments. The data given below were obtained by colleagues in Guangzhou using the equipment built by Dr. S. C. Wong.

2. 2. MEASUREMENTS OF FIELD MICROCLIMATE

A field environmental data acquisition system was linked to a microcomputer to record the output of 20 thermocouples for measurement of temperatures of individual leaves and soil, 2 infrared thermometers for canopy temperature measurement and 5 quantum sensors for incident

and canopy light environment. The data were acquired at 15 min intervals and stored daily. The equipment was set up in the paddy field of Guangdong Rice Research Institute, and was operated from early September to late November in 1986 to 1988. A near continuous record of microclimate in experimental plots of cv. Gui Chao-2 and Lemont was recorded.

Selected climatic data on canopy temperature and incident photon flux density (PFD) for six days in 1986 are shown in Figure 2.1. Although CDW-like conditions did not occur until after the sensitive grain filling period in 1986, representative weather patterns were observed. Examples of what may represent normal bright days (Oct. 16) and cloudy days (Oct. 18) in which mean daily temperature did not fall below 21 °C are shown. October 28 was a cloudy day in which canopy temperature rarely rose above 20 °C and probably represents WCDW event. October 30 was a bright day with canopy temperatures below 10 °C and a period of 2 h in the morning when canopy temperature rose from 10 °C to 15 °C, coincident with an increase in PFD from 500 to 1000 μmol photon m^{-2} s^{-1}. This pattern was considered representative of a DCDW event, and was taken as a model for the experimental treatments given below.

In 1988 the thermocouple temperature sensors were replaced with platinum resistance thermometers. Temperatures in air 2 m above the soil, in air at 60 cm in the canopy, and temperatures of the canopy (IR sensor), were monitored on a 15 minutes cycle. Temperature was also measured in the soil at 5 cm and 15 cm depth. Photosynthetically active radiation (400-700 nm) was again

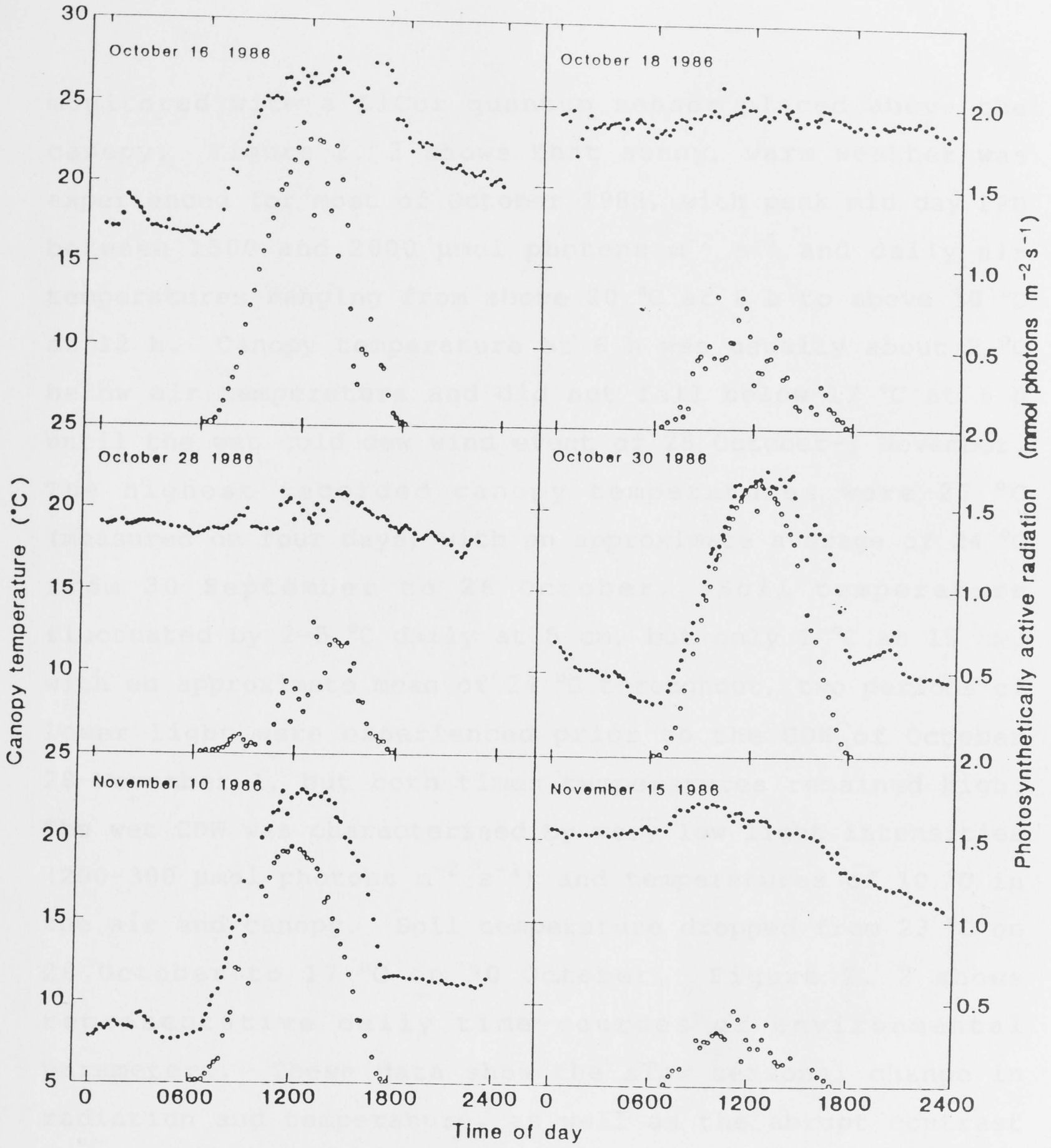

Figure 2. 1 Daily course of canopy temperature (●) and photosynthetically active radiation (○) in experimental plots of cv. Gui Chao-2 at the Rice Research Institute, Guangzhou.

monitored with a LiCor quantum sensor placed above the canopy. Figure 2. 2 shows that sunny, warm weather was experienced for most of October 1988, with peak mid day PFD between 1500 and 2000 μmol photons m^{-2} s^{-1} and daily air temperatures ranging from above 20 °C at 6 h to above 30 °C at 12 h. Canopy temperature at 6 h was usually about 2 °C below air temperature and did not fall below 17 °C at 6 h until the wet cold dew wind event of 28 October-1 November. The highest recorded canopy temperatures were 27 °C (measured on four days) with an approximate average of 24 °C from 30 September to 26 October. Soil temperature fluctuated by 2-5 °C daily at 5 cm, but only 1 °C at 15 cm, with an approximate mean of 24 °C throughout, two periods of lower light were experienced prior to the CDW of October 28-November 1, but both times temperatures remained high. The wet CDW was characterised by very low light intensities (200-300 μmol photons m^{-2} s^{-1}) and temperatures of 10 °C in the air and canopy. Soil temperature dropped from 23 °C on 28 October to 17 °C on 30 October. Figure 2. 2 shows representative daily time-courses of environmental parameters. These data show the slow seasonal change in radiation and temperature, as well as the abrupt contrast between the stable conditions (25 October), the CDW (31 October), and the return of bright light conditions (2 November).

These data were utilized to simulate the DCDW in controlled environment chambers and glasshouses, with single plant and canopy studies of photosynthesis in the laboratory in Canberra. In particular, Figure 2. 2 shows that soil temperature changes are strongly buffered compared with canopy temperature, and this was taken into account in

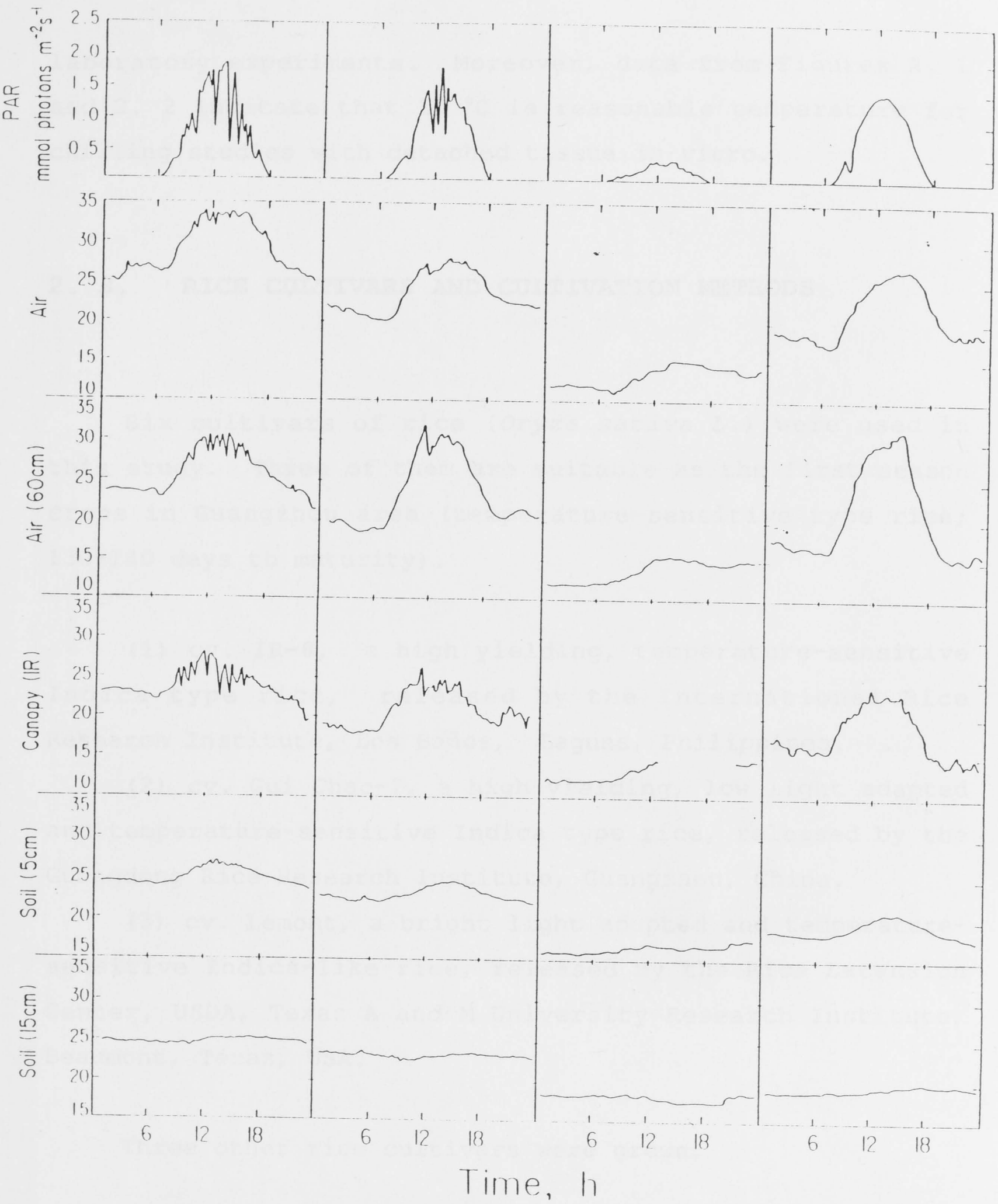

Figure 2. 2 Representative daily courses of micrometeorological parameters measured in the paddy field at the Rice Research Institute, Guangzhou, (1988).

laboratory experiments. Moreover, data from Figures 2. 1 and 2. 2 indicate that 10 °C is reasonable temperature for chilling studies with detached tissue *in-vitro*.

2. 3. RICE CULTIVARS AND CULTIVATION METHODS

Six cultivars of rice (*Oryza sativa L.*) were used in this study. Three of them are suitable as the first season crops in Guangzhou area (temperature-sensitive type rice; 130-140 days to maturity).

(1) cv. IR-8, a high yielding, temperature-sensitive Indica type rice, released by the International Rice Research Institute, Los Baños, Laguna, Philippines.

(2) cv. Gui Chao-2, a high yielding, low light adapted and temperature-sensitive Indica type rice, released by the Guangdong Rice Research Institute, Guangzhou, China.

(3) cv. Lemont, a bright light adapted and temperature-sensitive Indica-like rice, released by the Rice Extension Center, USDA, Texas A and M University Research Institute, Beaumont, Texas, USA.

Three other rice cultivars were grown.

(4) cv. Er Bai Ai, an Indica rice released by the Guangdong Rice Research Institute, Guangzhou, China, is suitable as the second season crop in Guangzhou area (photo-sensitive type rice; 135-145 days to maturity).

(5) cv. Hungarian-1, a Japonica-like rice, is an early maturing (90-100 days to maturity) variety characterised by

early seedling vigour, quick growth, and believed to be more chilling tolerant. This cultivar comes from Hungary and Romania, and was obtained from the Rice Research Laboratory, New South Wales Department of Agriculture, Yanco, Australia.

(6) cv. Calrose, a Japonica-like rice, believed to be more chilling tolerant, and adapted high light intensity. This cultivar comes from California, USA.

Seeds of the above six cultivars were soaked in water at room temperature (25 ^{o}C) for 24 hours, and then allowed to germinate on moist filter paper over soaked cotton wool in a covered crystallizing dish for 7-10 days. The dishes were kept in growth cabinets at 28-32 ^{o}C day / 22-25 ^{o}C night, with approximately 100 μmol photons $m^{-2}s^{-1}$ PFD from fluorescent and tungsten lamps, or kept in a normal glasshouse (32/25 ^{o}C day/night, shaded to 100 μmol photons $m^{-2}s^{-1}$) PFD.

Seedlings (5-6 per pot) were transferred into 120 mm diameter pots filled with vermiculite, standing in plastic trays of nutrient solution 100 mm deep (four pots per tray in early stages and two pots per tray later). The surface of the nutrient solution was covered with plastic foam beads to reduce evaporation. The plastic trays were covered with reflective Mylar or were inserted in reflective metal tubs to minimise heating of the roots. The nutrient solution, based on those used by Cook and Evans (1983), had the composition shown in Table 2. 1. Solutions were prepared freshly, adjusted to pH 4.5 with HCl, and replaced each week. The level of solutions was maintained by addition of tap water.

Table 2. 1. Nutrient solution used for rice growth in vermiculite

Nutrients	Stock solution ($g\ l^{-1}$)	Culture solution ($ml\ l^{-1}$)	Final concentration (mM)
Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) (1M)	236	2	2.0
Ammonium biphosphate ($NH_4H_2PO_4$) (1M)	115	0.5	0.5
Potassium nitrate (KNO_3) (1M)	101	3	3.0
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) (1M)	246.5	1	1.0
Fe EDTA (0.1M)	36.7	1	0.1
Micronutrients		1	——
Boric acid (H_3BO_3)	0.6		
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	0.4		
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	0.09		
Copper sulphate ($CuSO_4 \cdot 5H_2O$)	0.05		
Cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$)	0.025		
Molybdic acid ($H_2MoO_4 \cdot 4H_2O$)	0.02		

The newly imported rice cultivars, cv. Gui Chao 2, Er Bai Ai, and Lemont, were grown to maturity for seed under quarantine during the earliest experiments in the CSIRO Phytotron with natural daylight (1600 μmol photons $m^{-2}s^{-1}$, 32/25 °C day/night and high humidity throughout the summer and winter (December 1986 - August 1987). Seeds from these plants were used for experiments in the glasshouses and controlled environment growth chambers in the Research School of Biological Sciences, the Australian National University. All of the cultivars were grown in chambers controlled to 28/25 °C day(12h)/night(12h), approximately 80% relative humidity and 300-400 μmol photons $m^{-2}s^{-1}$ PFD from fluorescent tubes and tungsten lamps. These plants were used for studies of low temperature inhibition of photosynthesis in detached leaves.

Plants used for measurement of the canopy photosynthesis under simulated DCDW conditions were grown in the glasshouse, exposed to natural daylight in Canberra during summer and autumn (November 1987 - May 1988). At suitable stages potted mature plants were transferred into the controlled environment measurement chambers. Some plants were also kept in the same glasshouse under shade cloth which transmitted only 10% of incident external sunlight. These plants were used to study shade-sun acclimation of photosynthesis.

Plants that were used for single whole plant photosynthesis and yield measurement under DCDW simulation conditions were grown under natural daylight conditions in the glasshouse in Canberra during the summer and autumn

(November 1988 - May 1989). Environmental conditions in the natural daylight glasshouse were 35/22 °C day/night, with high relative humidity. On clear days, midday irradiation was approximately 1600 μmol photons $m^{-2}s^{-1}$. These plants were grown in larger pots (155 mm diameter, 600 mm height) filled with soil. One seedling was grown per pot, given one litre of nutrient solution per plant weekly, and were watered twice daily. At appropriate stages some plants were moved to the low temperature glasshouse for treatment, then returned to the control glasshouse until harvest.

Field studies were done at the Guangdong Rice Research Institute, Guangzhou, during September to November 1988. According to the traditional Chinese agricultural calendar, a Cold Dew Wind event could be expected on 8 - 22 October. A range of seven local cultivars and cv. Hungarian-1 were studied. Six of these have been ranked by GRRI in terms of chilling sensitivity (most sensitive = 6; least sensitive = 1) on the basis of effects on percentage of filled grains, as shows in Table 2. 2.

Three plantings of these cultivars were made at GRRI in June-August 1988 according to a schedule designed to bracket the timing of all previously recorded CDW events. Plots of 3.3 x 1.6 m were set out with each cultivar in randomized complete block design. The entire layout of plots was surrounded with border plantings. Each plot was planted with a single plant per hill at transplanting, spaced 20 cm between rows and 17 cm between columns. The plots had been prepared with 750 kg ha^{-1} of pigs manure and

Table 2. 2 Ranking of differences in chilling sensitive rice cultivars using in field experiments, Guangzhou

Cultivars	Sensitivity
Er Zao Zao (EZZ)	1
Huang Ke Heng Ye (HKHY)	1
Er Bai Ai (EBA)	2
Bao Xuan-2 (BX-2)	3
Hua Quan (HQ)	5
Wan Zhu-3 (WZ)	6
Gui Chao-2 (GC-2)	sensitive
Hungarian-1 (H-1)	tolerant

375 kg ha-1 of peanut cake, and were managed by normal cultivation techniques. At the time of study this meant that water was provided to the plots when judged to be required.

At the same time, potted plants of these cultivars were grown next to the plots in the field. Pots (20 cm diameter, 33 cm height) were filled with soil obtained from the same field, and five single seedlings were grown per pot. They were given a basal manure treatment of peanut cake, 1.41g; $(NH_4)_2SO_4$, 0.47g; KCl, 0.47g; and $Ca(H_2PO_4)_2 \cdot H_2O \cdot CaSO_4 \cdot 2H_2O$, 0.94g each pot. Additional nutrients were given according to growth performance of plants, which were watered twice daily. In mid September, one set of 5 pots of each cultivar was transferred to a controlled environment chamber set at 30 °C day/25 °C night, with 10 h PFD of 700 μmol photons m^{-2} s^{-1} from tungsten and fluorescent lamps. These plants were kept as controls against a natural CDW event which could be expected middle or late October. At appropriate stages some plants were transferred to the simulated CDW conditions in controlled environmental chambers for stress treatment, as described in Chapter 5.

2. 4. PHOTOSYNTHETIC MEASUREMENT METHODS

2. 4. 1. Measurements of Maximum Photosynthesis and Quantum Yield by O_2 Exchange

In most experiments, the light response of photosynthetic O_2 evolution in leaf segments was measured at

CO_2 saturation in a leaf disc O_2 electrode (Hansatech Ltd, Kings Lynn, Norfolk UK). The oxygen electrode is a specially designed form of an electrochemical cell in which a current flows proportional to the activity of oxygen present in the gas phase (Delieu and Walker, 1983). The electrode dome is inserted through an aperture in the floor of the temperature controlled leaf chamber. Oxygen diffuses through a thin teflon membrane over the electrode which is bathed in electrolyte buffer (0.2mM H_3BO_3 with saturated KCl, and 1.0mM potassium carbonate ($NaHCO_3$) at pH 9.0. The chamber was flushed with N_2 to remove all the O_2, thereby establishing an electrode output (residual current) at zero mb O_2 (Delieu and Walker, 1983).

Leaf segments (60-80 mm) were cut from the leaf lamina, and were quickly aligned side by side, with tape at the ends, and a disc of 10 cm^2 cut from parallel leaf segments. The disc was reassembled on moist cloth matting in the chamber of the electrode which was closed and calibrated by injecting 1 ml of air from the gas-tight syringe. The increase in signal brought about by a corresponding increase in the partial pressure of oxygen at the cathode was used to calculate the effective volume of the chamber and the linearity of the electrode response (Delieu and Walker, 1972, 1983).

The chamber was filled with 10-15% CO_2 by the operator breathing gently into the chamber, as described by Adams *et al.*, (1986). The mean CO_2 concentration used in the quantum yield measurements was 14.9 ± 0.6% (n=85). The quantum yield measured in this way represents the maximum value for net CO_2 uptake in photosynthesis because, at this CO_2 concentration, stomatal diffusion is not limiting, and photorespiration is completely inhibited. The maximum rate

of photosynthesis measured in this way is not inhibited by the high CO_2 concentration, as was shown by Terashima (see Figure 2. 3). The CO_2 response curve for photosynthetic O_2 evolution in leaves of cv. IR-8 is shown in Figure 2. 3 and the maximum rate, of 35 μmol m^{-2} s^{-1}, is comparable with that routinely observed at light saturation in experiments using CO_2 exchange techniques (Makino et al, 1987).

Prior to measurement of quantum yield, leaves were twice illuminated for 5 minutes (with 1 min dark interval between) with broad band blue light (220 μmol photons m^{-2} s^{-1}) for observation of room temperature fluorescence transients and elimination of induction phenomena. The blue filter was then removed and light response curves of photosynthetic O_2 evolution were measured using a fan-cooled quartz iodide lamp source and a regulated switching power supply (Power/Mate, Hackensack, New Jersey 97601, USA). The maximum output of a 50W/12V lamp was 800 μmol photons m^{-2} s^{-1} PFD (400-700nm), and with a 100W/12V was 1600-2000 μmol photons m^{-2} s^{-1}. Neutral density filters (Melles Griot, Amsterdem) were used to change PFD, and the incident PFD was measured with a Li-Cor quantum meter (Model LI-188B with LI-190SB quantum sensor). The rate of O_2 evolution was allowed to stabilise (about 4-5 minutes) at each light intensity in the sequence of measurement from darkness to maximum light. Quantum yield was estimated from the initial slope of the light response curve, after correction for reflectance and transmittance of the leaf tissues which were measured in a small Ulbricht sphere with standardized reflectance cards (Kodak) of 4.3%, 17.8%, and 93.3%, and using the same light source.

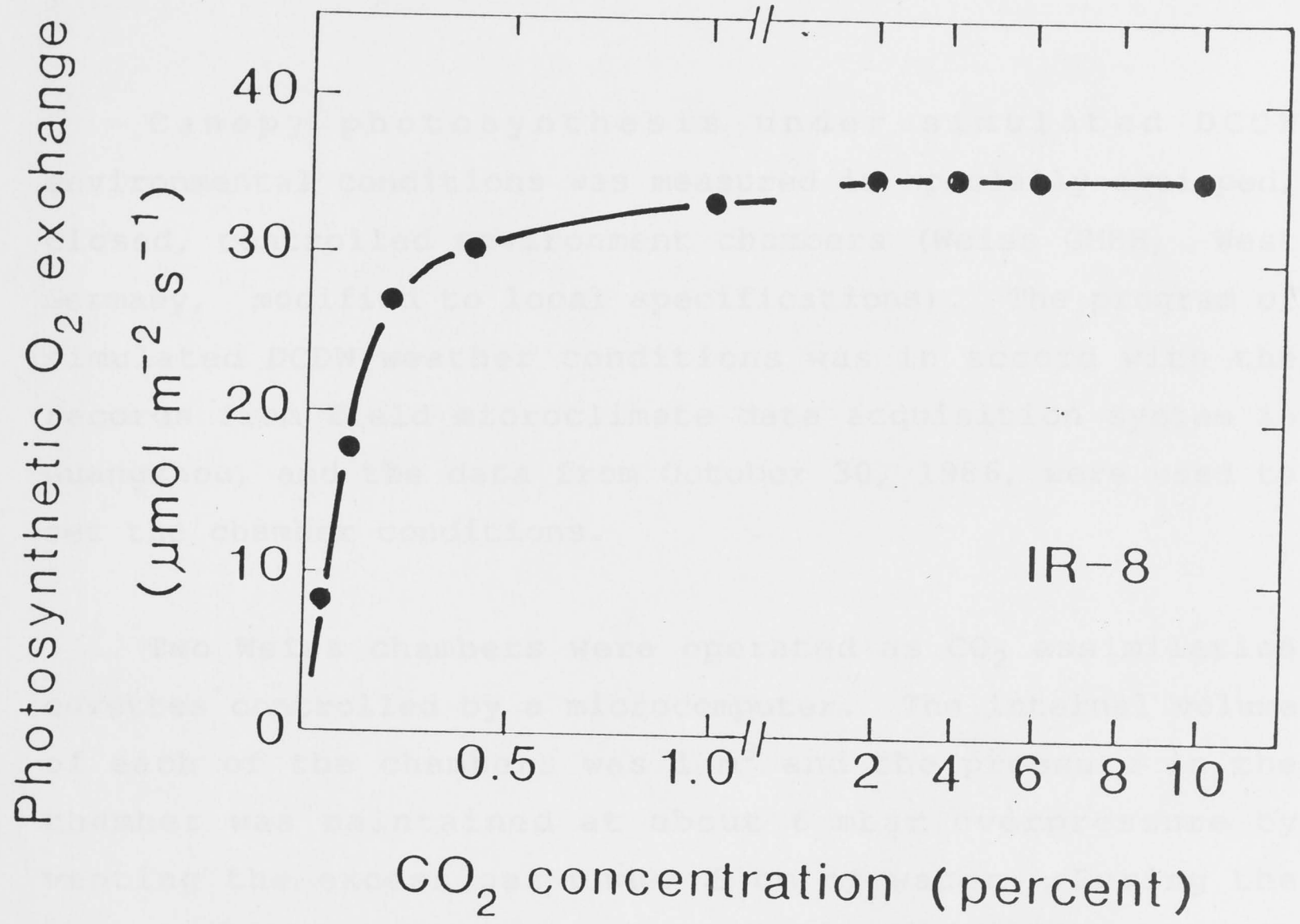

Figure 2. 3 The CO_2 response curve of photosynthetic O_2 evolution in rice , cv. IR-8, measured in the leaf disc O_2 electode at 500 µmol photons m^{-2} s^{-1}. Plants were grown in a controlled environment cabinet (300-400 µmol photons m^{-2} s^{-1}). (Data of Terashima in Huang *et al.* 1989b).

2. 4. 2. Measurement of Canopy Photosynthesis in a Closed, Controlled Environment Chamber

Canopy photosynthesis under simulated DCDW environmental conditions was measured in specially-equipped, closed, controlled environment chambers (Weiss GMBH, West Germany, modified to local specifications). The program of simulated DCDW weather conditions was in accord with the records from field microclimate data acquisition system in Guangzhou, and the data from October 30, 1986, were used to set the chamber conditions.

Two Weiss chambers were operated as CO_2 assimilation cuvettes controlled by a microcomputer. The internal volume of each of the chambers was 1 m^3 and the pressure in the chamber was maintained at about 6 mbar overpressure by venting the excess gas under 6 cm of water. During the photoperiod each chamber was pressurized with CO_2 -free air at a flow rate of 8 L min^{-1} through a mass flow controller and CO_2 partial pressure (340 μbar) was maintained by injecting pure CO_2 through the mass flow controllers. Two chambers were monitored alternatively using an absolute infrared CO_2 analyser (Beckman model 864, Fullerton, California, USA). The rates of pure CO_2 injection into the chambers were determined by the microcomputer, based upon the partial pressures of CO_2 and CO_2 assimilation rates of the preceding scanning period, and the predicted CO_2 assimilation rates for the next scanning period. Rates of CO_2 assimilation were calculated as the difference between the flow rates of CO_2 into the chambers and the amount of CO_2 replaced by the injection of CO_2-free air and CO_2. The

change in CO_2 partial pressure within the chambers was also taken into consideration. During the dark period, CO_2 injection was stopped and flow rate of CO_2-free air were increased from the minimum flow rates of 8 L min^{-1} to compensate for the increase in CO_2 partial pressure due to respiration. Respiration was also calculated. The control system was able to maintain the partial pressure of CO_2 within ± 2 μbar of the set partial pressure.

2. 4. 3. Measurement of Single Plant Photosynthesis in an Open Chamber System

Comparison of photosynthetic characteristics of canopies with single whole plants under simulated CDW stress conditions, was made using a single plant gas exchange open system based on the ventilated chamber technique (Greenwood *et al.*, 1981; Wong and Dunin, 1987). The frame of the chamber was made of aluminium alloy, and the chamber wall was lined with 100 μm thick transparent polyethylene film. The volume of the chamber was 0.3 m^3, and the potted plant was held with an airtight seal in the base. Thermocouples were fitted for the measurement of individual leaf, air, and soil temperatures, and humidity was also measured. Air was impelled from the base of chamber by a tangential blower at a flow rate of about 600 L min^{-1}, with an air outlet at the top of the chamber. There was no temperature control in the chamber, but relatively high air flow rate and transpiration from leaves maintained air temperatures which were only 3 - 4 °C higher within the chamber than outside.

The application of a thermal mass flowmeter (Model FC-260, Tylan, Torrance, CA) to measure the photosynthetic gas exchange fluxes increased the potential accuracy of determination of assimilation rates. This controller was used to inject CO_2 at a small, known flow rate, and the increased CO_2 concentration was used to calculate air flow through the chamber. The gas analysis samples were taken from two 5 mm bore plastic tubes in the inlet and outlet air ducts (100 mm diameter) in the chamber bottom. A diaphragm pump was used to send samples to an infra-red gas analyser (IRGA) for measurement. Carbon dioxide partial pressure at the inlet of the chamber was measured with an absolute IRGA (Binos 1, Leybold Heraeus, West Germany). Differential partial pressure of CO_2 between the inlet and outlet was determined with a differential CO_2 IRGA (Binos 1). Water vapor pressure was measured with an absolute water vapor IRGA (Binos 1). The calculation of photosynthetic rate and transpiration rate was described in Wong *et al.* (1978).

2. 5. CHLOROPHYLL FLUORESCENCE MEASUREMENT METHODS

2. 5. 1. Chlorophyll Fluorescence Measurement at Room Temperature

In early experiments, chlorophyll fluorescence from rice leaves at 25 °C in the O_2 electrode chamber was measured with a Hansatech fluorescence detector after 10-20 min dark-adaptation. The maximum fluorescence (F_p) was measured immediately after illumination with blue light (PFD 300 μmol photons m^{-2} s^{-1}, 320-550nm). The final

fluorescence (F_t) was measured at the end of a second 5 min period in blue light, with 1 min dark between light periods. Variable fluorescence (F_v) was calculated as $F_v = F_p - F_t$ (Figure 2. 4).

Recent developments in room temperature fluorescence techniques (Schreiber *et al.*, 1985, 1986) have provided new instruments which employ a special measuring system based on a pulsed light-emitting-diode (650 nm) for fluorescence excitation in which only the resulting pulsed fluorescence signal is amplified by a highly selective amplifier system. This system, the PAM chlorophyll fluorimeter (Walz, Effeltrich, West Germany) was used to measure initial fluorescence (F_o) of dark adapted leaf discs after exposure to weak pulsed red light. Maximum fluorescence (F_m) was measured during a 1 s flash of saturating white light. Variable fluorescence (F_v) was calculated as $F_v = F_m - F_v$.

2. 5. 2. Chlorophyll Fluorescence Measurement at 77 K Temperature

Chlorophyll fluorescence was also measured in leaf discs frozen to 77 K, the temperature of liquid N_2. The principle of the method was based on Powles and Björkman (1982), using an instrument constructed by S.C. Wong, similar to that described by Osmond *et al.* (1989). Leaf discs were dark adapted at the end of a 10 mm diam quartz in a brass chamber. The chamber was fitted to a fibre optic cable of the fluorimeter and partly submerged in liquid N_2. After freezing, fluorescence was excited by weak blue light (PFD ~ 1 μmol photons m^{-2} s^{-1}, 450 nm broad band blue

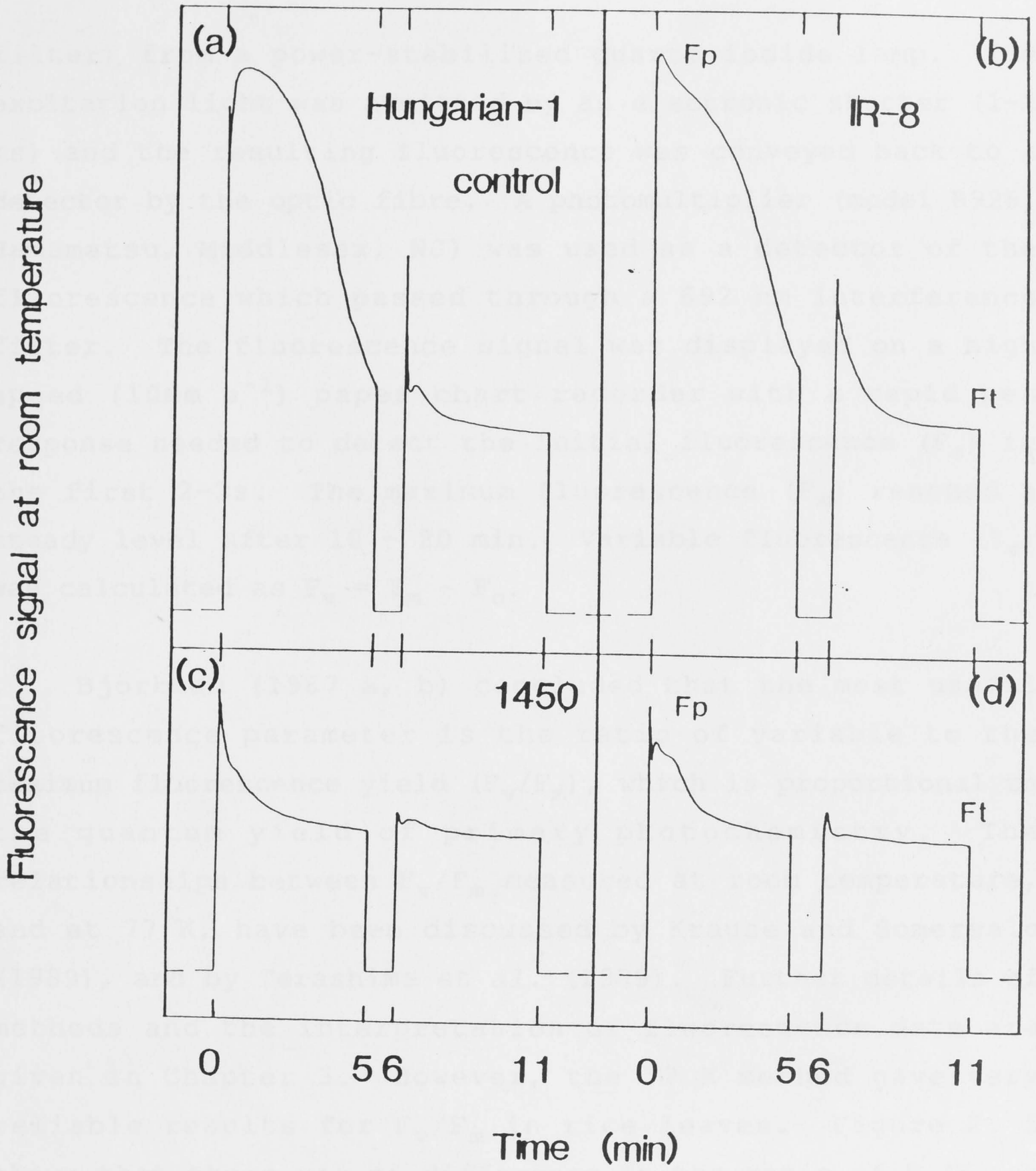

Figure 2. 4 Recorder tracings of chlorophyll fluorescence measured at room temperature in the O_2 electrode chamber with a Hansatech fluorescence detector. (a) and (b) control leaves; (c) and (d) photoinhibited leaves. The photoinhibitry treatments involved exposing leaves at 1450 µmol photons m^{-2} s^{-1} for 9 h at room temperature. F_p and F_t denote, maximum fluorescence and final fluorescence, respectively.

filter) from a power-stabilized quartz iodide lamp. The excitation light was admitted by an electronic shutter (1-2 ms) and the resulting fluorescence was conveyed back to a detector by the optic fibre. A photomultiplier (model R928, Hamamatsu, Middlesex, NJ) was used as a detector of the fluorescence which passed through a 692 nm interference filter. The fluorescence signal was displayed on a high speed (10mm s^{-1}) paper chart recorder with a rapid pen response needed to detect the initial fluorescence (F_o) in the first 2-3s. The maximum fluorescence (F_m) reached a steady level after 10 - 20 min. Variable fluorescence (F_v) was calculated as $F_v = F_m - F_o$.

Björkman (1987 a, b) concluded that the most useful fluorescence parameter is the ratio of variable to the maximum fluorescence yield (F_v/F_m), which is proportional to the quantum yield of primary photochemistry. The relationships between F_v/F_m measured at room temperature, and at 77 K, have been discussed by Krause and Somersalo (1989), and by Terashima *et al.* (1989). Further details of methods and the interpretation of fluorescence data are given in Chapter 3. However, the 77 K method gave very reliable results for F_v/F_m in rice leaves. Figure 2. 5 shows that there was no difference in the ratio of F_v/F_m at different positions along the three functional leaves of mature rice plants.

2. 6. THE ESTIMATION OF CHLOROPHYLL CONCENTRATION

The chlorophyll concentration of rice leaves was estimated by extracting small leaf discs (5 x 0.1 cm^2) in 1.5 ml N,N-dimethylformamide (DMF) which was stored in a

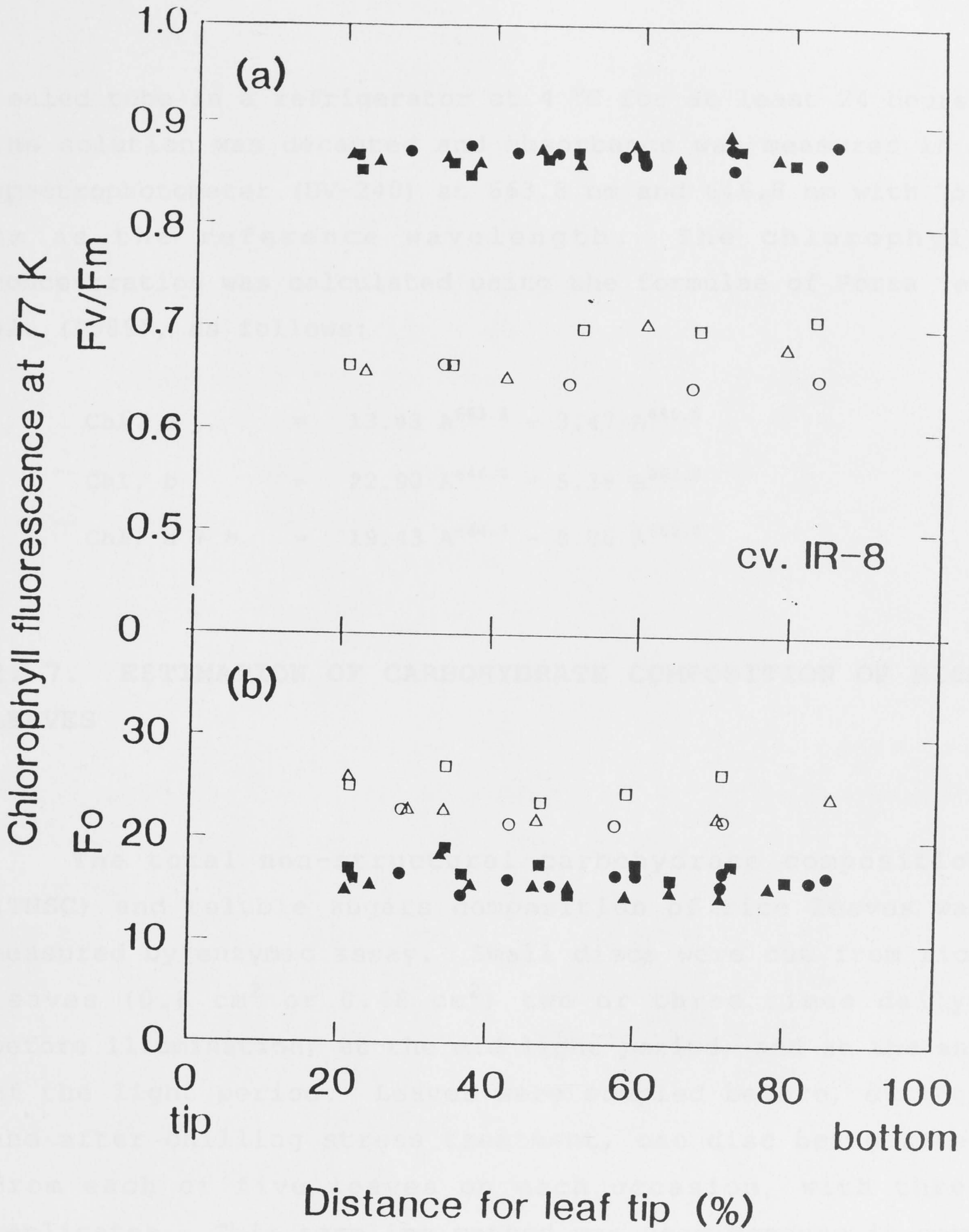

Figure 2. 5 Initial fluorescence, F_o, and the ratio between variable and maximum fluorescence, F_v/F_m, with distance from the tip to ligule of three functional leaves of mature rice plants (cv. IR-8). Symbols, control (closed symbols), low temperature photoinhibition (2000 μmol photons m^{-2} s^{-1}, 10 °C for 2 h) treatment (open symbols), (●,○), (▲,△), and (■,□) represent flag, second and third leaf, respectively.

sealed tube in a refrigerator at 4 °C for at least 24 hours. The solution was decanted and absorbance was measured in a spectrophotometer (UV-240) at 663.8 nm and 646.8 nm with 750 nm as the reference wavelength. The chlorophyll concentration was calculated using the formulae of Porra *et al.* (1989), as follows:

$$\text{Chl. } a = 13.43\ A^{663.8} - 3.47\ A^{646.8}$$

$$\text{Chl. } b = 22.90\ A^{646.8} - 5.38\ A^{663.8}$$

$$\text{Chl. } a + b = 19.43\ A^{664.8} - 8.05\ A^{663.8}$$

2. 7. ESTIMATION OF CARBOHYDRATE COMPOSITION OF RICE LEAVES

The total non-structural carbohydrate composition (TNSC) and soluble sugars composition of rice leaves was measured by enzymic assay. Small discs were cut from rice leaves (0.8 cm^2 or 0.48 cm^2) two or three times daily, before illumination, at the mid light period, and at the end of the light period. Leaves were sampled before, during, and after chilling stress treatment, one disc being taken from each of five leaves on each occasion, with three replicates. This sampling method was used because it gave more reliable results. The samples were immersed in liquid N_2 and ground to a fine powder with PVC rods in small glass tubes, which were stored in a -80 °C freezer until extraction. Samples were extracted by adding 1.5 ml of distilled water to each vial, which was capped with a marble and kept in a boiling water bath for about 20 min. After cooling, 50 µl of the supernatant was withdrawn for analysis of soluble sugars, and the remainder used for the estimation of TNSC.

The soluble sugars sample was treated with 125 µl invertase at room temperature for about 30 min, centrifuged for 5 min in an Eppendorf Micro Centrifuge at 15000 r.p.m., and 20-50 µl of the supernatant taken for assay with glucose reagent. The remainder of the original extract was treated with 0.55 ml of a starch hydrolyzing enzyme (clarase 900) and incubated for 24 h in the water bath at 37 °C. After cooling again, the sample was centrifuged, and a 20-50 µl of the supernatant was withdrawn for estimation of TNSC, with the glucose reagent. Starch was calculated by subtracting soluble sugars from TNSC.

Clarase 900 (Miles Laboratories) is a mixture of amylases including glucoamylase, exo-1,4-α-glucosidase, and endoamylase, 1,4-α-D-glucan glucohydrolase. The endoamylase breaks the α-1,4 bonds of the amylose in starch, and glucoamylase with exo-1,4-α-glucosidase which then breaks the α-1,6 bonds, decomposing starch to glucose. Clarase 900 was dissolved in 0.2 N sodium acetate buffer, pH 4.6 (0.05 g ml^{-1}), and was dialysed against the same buffer for 24 hours in a cold room at about 4 °C, then diluted to 0.02 g ml^{-1} before use. The hydrolysis reaction is shown in the following equation:

$$\text{Starch} \xrightarrow[\text{glucohydrolase}]{\text{1,4-α-D-Glucan}} \text{Glucose} + \text{Maltose} \xrightarrow[\text{glucoamylase}]{\text{exo-1,4-α-Glucosidase}} \text{Glucose}$$

Invertase (Saccharase; β-Fructfuranosidase; β-D-fructofuranoside fructohydrolase; Sigma Chemical Company) was diluted to 5 µg ml^{-1} in 0.2 N acetate buffer, pH 4.6, before use. This enzyme hydrolyses sucrose as follows:

$$\text{Sucrose} \xrightarrow{\text{invertase}} \text{Glucose} + \text{Fructose}$$

Glucose reagent, (Behring Diagnostics Inc.) containing 40 units of hexokinase and glucose-6-phosphate dehydrogenase with 4 mM Mg^{2+}, 1.5 mM ATP, 3.2 mM NADP, and 125 mM Hepes buffer, pH 7.8, was dissolved in 50 ml distilled water. Phosphoglucose isomerase (10 µl, approx. 100 units; Sigma Chemical Company) was added before use. These coupled enzymes permit estimation of hexoses and hexose phosphates as follows:

$$\text{Glucose} + \text{Fructose} + 2\text{ATP} \xrightarrow{\text{hexokinase}} \text{6-P-Glucose} + \text{6-P-Fructose} + 2\text{ADP}$$

$$\text{6-P-Fructose} \xrightarrow{\text{P-glucoisomerase}} \text{6-P-Glucose}$$

$$2\ \text{6-P-Glucose} + 2\text{NADP}^{+} \xrightarrow[\text{dehydrogenase}]{\text{6-P-Glucose-}} 2\ \text{6-P-Gluconate} + 2\text{NADPH}$$

Aliquots of 1 ml of this reagent were added to cuvettes and the initial absorbance was read at 340 nm in a spectrophotometer (Shimadzu, UV-240). Then 20 - 50 µl of the extracted sample was added to each cuvette and allowed to react with the reagent for 5 - 10 min and the final absorbance reading at 340 nm was taken. Each batch of assays included 5 glucose standards (20 - 50 µg) as well as blanks for extracts and reagents. The glucose equivalents

were calculated from the change in absorbance due to NADPH production as shown in above equations. Data were corrected for leaf area of the samples used, and soluble sugars or starch were expressed as gm^{-2} (glucose).

Figure 2. 6 shows the contents of soluble sugars and starch in the different positions of the lamina of the productive leaves (flag, second, and third from top) of rice when samples were collected at the same time. There was some variation in soluble sugars towards the base of the third leaf, but the mean values in different leaves were similar. Figure 2. 7 shows that the concentrations of soluble sugars and starch changed throughout a normal day, with values at the end of the day being higher than in the morning.

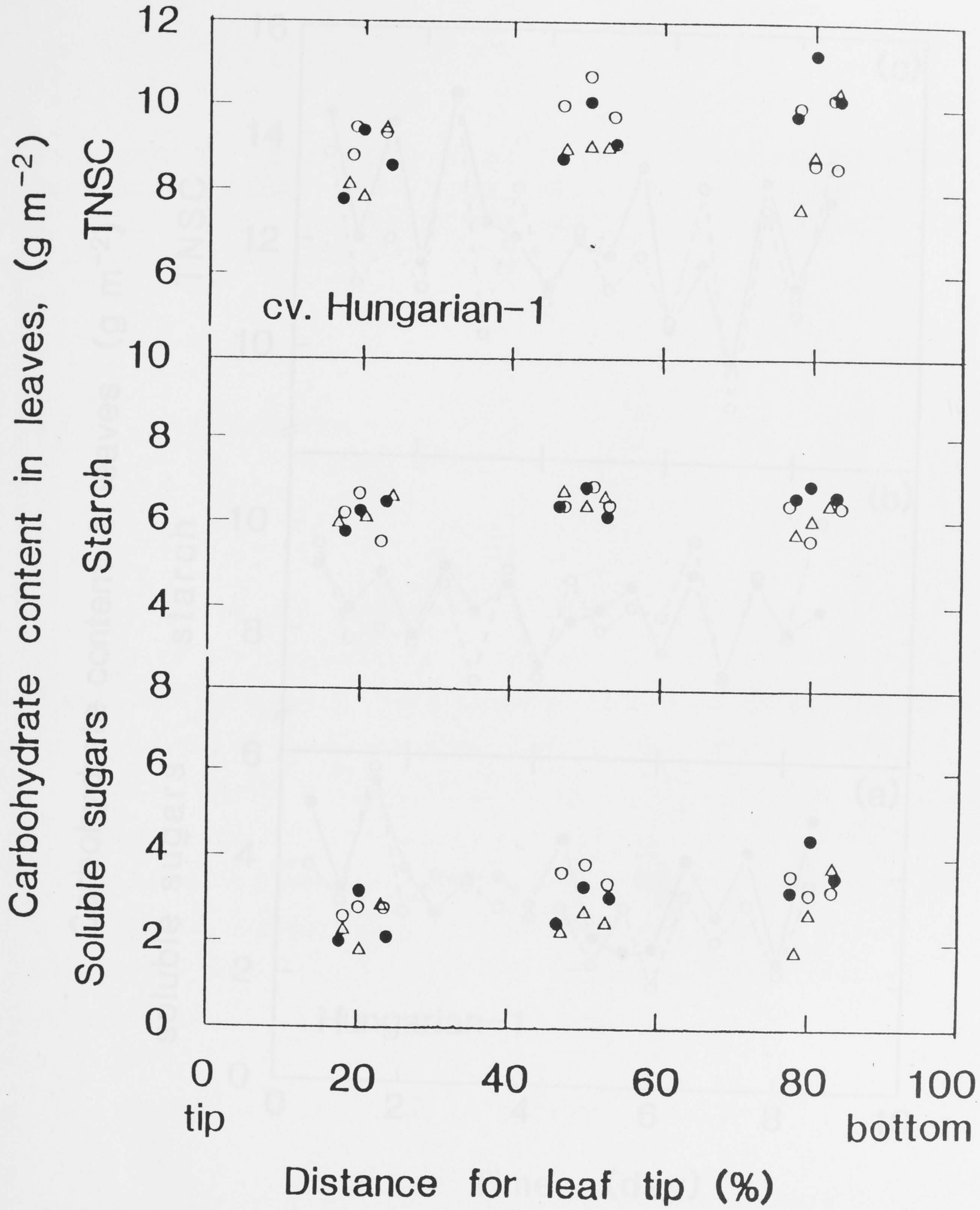

Figure 2. 6 Soluble sugars, starch and TNSC contents in different positions of the lamina of flag, second and third leaves of cv. Hungarian-1. Symbols ●, ○ and △ represent flag, second and third leaf, respectively.

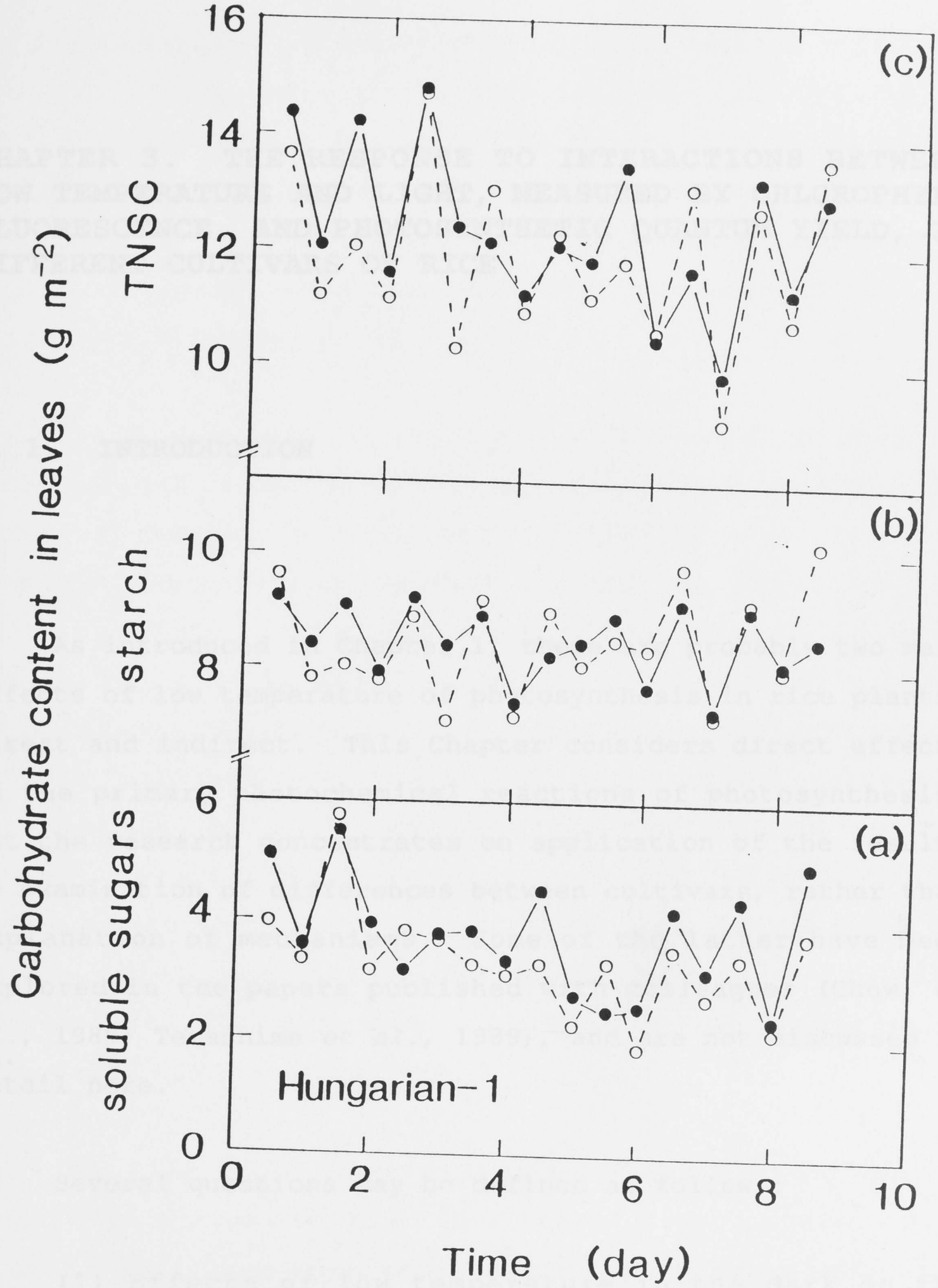

Figure 2. 7 Soluble sugars, starch and total non-structural carbohydrate (TNSC) contents in flag and second leaves of cv. Hungarian-1 during a 9 day period. Symbols ●, and ○ represent flag and second leaf, respectively.

CHAPTER 3. THE RESPONSE TO INTERACTIONS BETWEEN LOW TEMPERATURE AND LIGHT, MEASURED BY CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHETIC QUANTUM YIELD, IN DIFFERENT CULTIVARS OF RICE

3. 1. INTRODUCTION

As introduced in Chapter 1, there are probably two main effects of low temperature of photosynthesis in rice plants, direct and indirect. This Chapter considers direct effects on the primary photochemical reactions of photosynthesis, but the research concentrates on application of the results to examination of differences between cultivars, rather than explanation of mechanisms. Some of the latter have been explored in the papers published with colleagues (Chow, *et al.*, 1989; Terashima *et al.*, 1989), and are not discussed in detail here.

Several questions may be defined as follows:

(1) Effects of low temperature in the dark on the primary photochemical reactions. It is generally agreed that prolonged exposure of leaves to low temperature in the dark leads to changes in the primary photochemical reaction activity, due to reduction of PS II water splitting activity, (Smillie and Nott, 1979; Terashima *et al.* 1989). This response has been extensively studied by means of room

temperature fluorescence, and was the central point of research done by my colleagues in this project (Smillie *et al.* 1988). For this reason, I did not give much attention to this question. However, it should be noted that rice (cv. IR-8) is not very sensitive to prolonged exposure to 0 °C in the dark, when compared with cucumber (Smillie *et al.*, 1988; Terashima *et al.*, 1989).

(2) Effects of low night temperature on primary photochemical processes of photosynthesis under bright light at normal temperature during the following day. That is, does low temperature in the dark increase the sensitivity of photosynthesis to photoinhibition? Under natural conditions, this is often the way plants are first exposed to chilling (see Figure 2. 1). Low night temperature is usually associated with clear sky conditions, which may continue the next day. Unfortunately, there are no reports in the literature which specifically study this problem. If this is important, one could imagine that damage to PS II water splitting reactions (see above) would make photosynthesis more sensitive to bright light, leading to more damage to photochemistry.

(3) Effects of low temperature in the light on the primary photochemical reactions. Low night temperature is usually followed by several hours low temperature with bright light the next day (see Figure 2. 1). There are many studies which show that low temperature exaggerates photoinhibition (Powles *et al.*, 1983; Ögren and Öquist, 1984 a, b; Öquist *et al.*, 1987). Although rice is less sensitive to this interaction of low temperature with bright light (Terashima *et al.*, 1989), it is possible that cultivars

differ in this response, and the research described in this Chapter concentrates on this problem. Previous studies show that the reaction centre of PS II is the primary site of damage under this condition (Chow *et al.*, 1989), so that one should be able to use chlorophyll fluorescence and photosynthetic quantum yield to indicate differences between cultivars.

(4) Effects of low temperature on recovery from photoinhibition. It is well known that the extent of photoinhibition is dependent on the rate of damage and the rate of recovery. Greer *et al.*, (1986); (1988) and Greer and Laing (1988 a,b have studied this response in detail. Thus, it is possible that differences between cultivars in their sensitivity to low temperature and bright light depend on differences in recovery processes. At least two possibilities need to be studied. Firstly, low night temperature may slow down recovery from photoinhibition at normal warm day temperatures. Secondly, low temperature in the light could reduce the rate of recovery during photoinhibition itself. These possibilities may be useful for screening differences between cultivars, and should be considered in any program.

Because the irradiance and temperatures throughout the whole growth of the plants can have a large effect on the subsequent susceptibility to the interaction between light and temperature, in this research, I found it necessary to characterise the light responses of rice cultivars in detail. There are relatively few data on the photosynthetic responses to light in rice plants suited to physiological analysis (Akita *et al.*, 1968; Tu *et al.*, 1988). In this

Chapter, differences in the characteristics of low temperature and bright light effects on primary photochemical reactions in photosynthesis of rice cultivars will be described using techniques of chlorophyll fluorescence and quantum yield. In later Chapters, I will examine if these effects on the primary photochemical processes are responsible for reduced photosynthesis and reduced grain yield under Cold Dew Wind conditions.

3. 2. MATERIALS AND METHODS

In all experiments, plants were grown as described in Section 2. 3. Only the mid-section of the lamina of the functional leaves (flag, second and third leaves) from mature rice plants, collected during heading/flowering, was used for measurement. In the earliest experiments, it was shown that the fluorescence properties of the middle portion of the lamina from different leaves were sufficiently uniform for comparisons (Figure 2. 5). The lowest temperature used in these experiments was 10 °C, consistent with night temperature measured in the field (Figure 2. 1)

Two types of experiments were done. In the first, attached leaves of potted plants were exposed to different light intensity treatments in air for differing periods. Five or six leaves were inserted into a leaf holder between nylon threads to maintain the laminae in a horizontal array under a water cooled xenon-arc lamp. The leaf temperature

during these photoinhibition treatments was 25 - 30 °C. When these plants were pretreated in the dark in the environmental controlled growth cabinet at 10 °C for 12 hours, the pots were immersed in a 20 °C water bath to avoid subsequent water stress effects due to cold roots (McWilliam *et al.*, 1982). The second type of treatment employed detached leaf segments or leaf discs, which were floated on plastic trays of tap water, which were set in a water bath and maintained at 10 °C or 25 °C. The leaf pieces were either illuminated with the xenon-arc lamp, or kept in the dark.

Light response curves of leaf photosynthesis were measured by O_2 exchange at CO_2 saturation in the leaf disc electrode as described in Section 2. 4. 1. Chlorophyll fluorescence was measured as described in Sections 2. 5. 1 and 2. 5. 2.

3. 3. EXPERIMENTS AND RESULTS

3. 3. 1. Light responses of photosynthetic O_2 exchange

The first goal of these studies was to discover if rice cultivars differ in response to light. This is of basic importance because it is well known that shade adapted plants are more sensitive to photoinhibition than sun adapted plants (Adams, 1988; Anderson and Osmond, 1987). Such differences between rice cultivars were indicated by Tu

et al. (1988), and these could confuse the interpretation of low temperature and light experiments.

There was a substantial decrease in the rate of light saturated photosynthesis when rice plants were transferred from full sunlight to a shaded enclosure (10 % of full sunlight) in the glasshouse for two weeks (Figure 3. 1). However, the photosynthetic quantum yield was not different between sun and shaded plants, and 77 K fluorescence properties were unchanged (Table 3. 1). According to Tu *et al.* (1988), the cultivars used in these experiments should show the properties of a shade plant (cv. Gui Chao-2) and a sun plant (cv. Lemont), respectively. However, under our conditions, there was no difference.

Extensive comparisons were made of the sensitivity to photoinhibition in leaves of four rice cultivars grown under similar conditions in the CSIRO phytotron glasshouses. Figure 3. 2 shows that exposure of leaves to 1450 μmol photons m^{-2} s^{-1} in air for 9 h at 25 °C, led to reduction of the initial slope (quantum yield) and maximum rate of photosynthesis in three cultivars which had been grown in full sunlight. Quantum yields (mol O_2 mol^{-1} absorbed photons), before and after treatment, were 0.095, 0.064 (Hungarian-1), 0.098, 0.062 (IR-8), and 0.096, 0.056 (Lemont), respectively.

The above changes due to photoinhibition at 25 °C were time and light intensity dependent, but there were no differences between cultivars. Figure 3. 3 shows results from 86 experiments with attached leaves of four cultivars

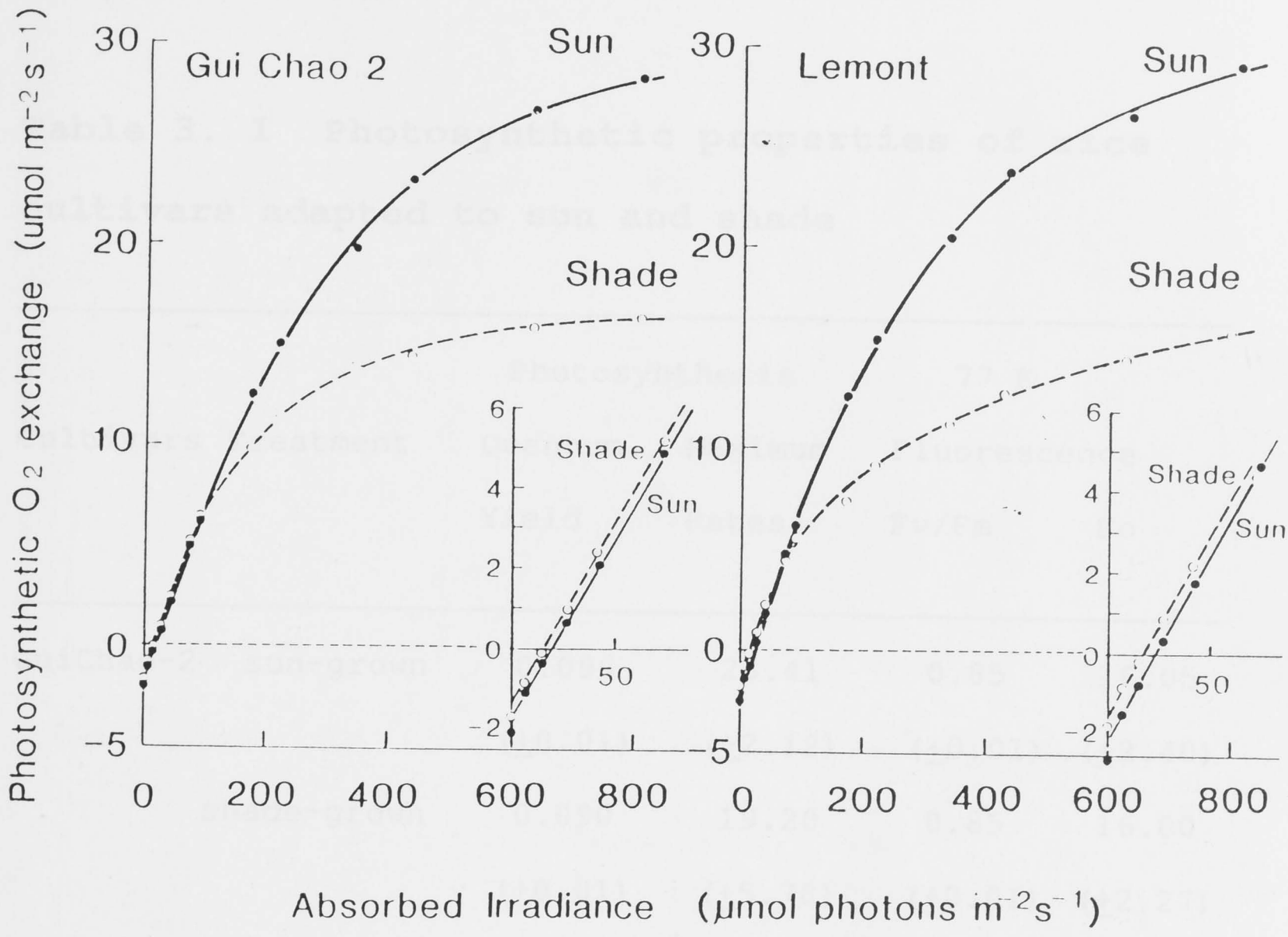

Figure 3. 1 Light response curves measured in the early morning using leaves of rice cultivars grown in the glasshouse in full sunlight (●) or 10% sunlight (○). The inserts show the guantum yield region of the response curve.

Table 3. 1 Photosynthetic properties of rice cultivars adapted to sun and shade

Cultivars	Treatment	Photosynthesis		77 K Fluorescence	
		Quantum Yield	Maximum Rates	Fv/Fm	Fo
GuiChao-2	sun-grown	0.090	28.41	0.85	16.05
		(±0.01)	(±2.12)	(±0.01)	(±2.40)
	shade-grown	0.090	19.20	0.85	16.00
		(±0.01)	(±5.78)	(±0.01)	(±2.27)
Lemont	sun-grown	0.093	29.15	0.86	17.73
		(±0.01)	(±2.38)	(±0.01)	(±1.73)
	shade-grown	0.088	17.73	0.85	17.85
		(±0.01)	(±3.23)	(±0.01)	(±2.26)

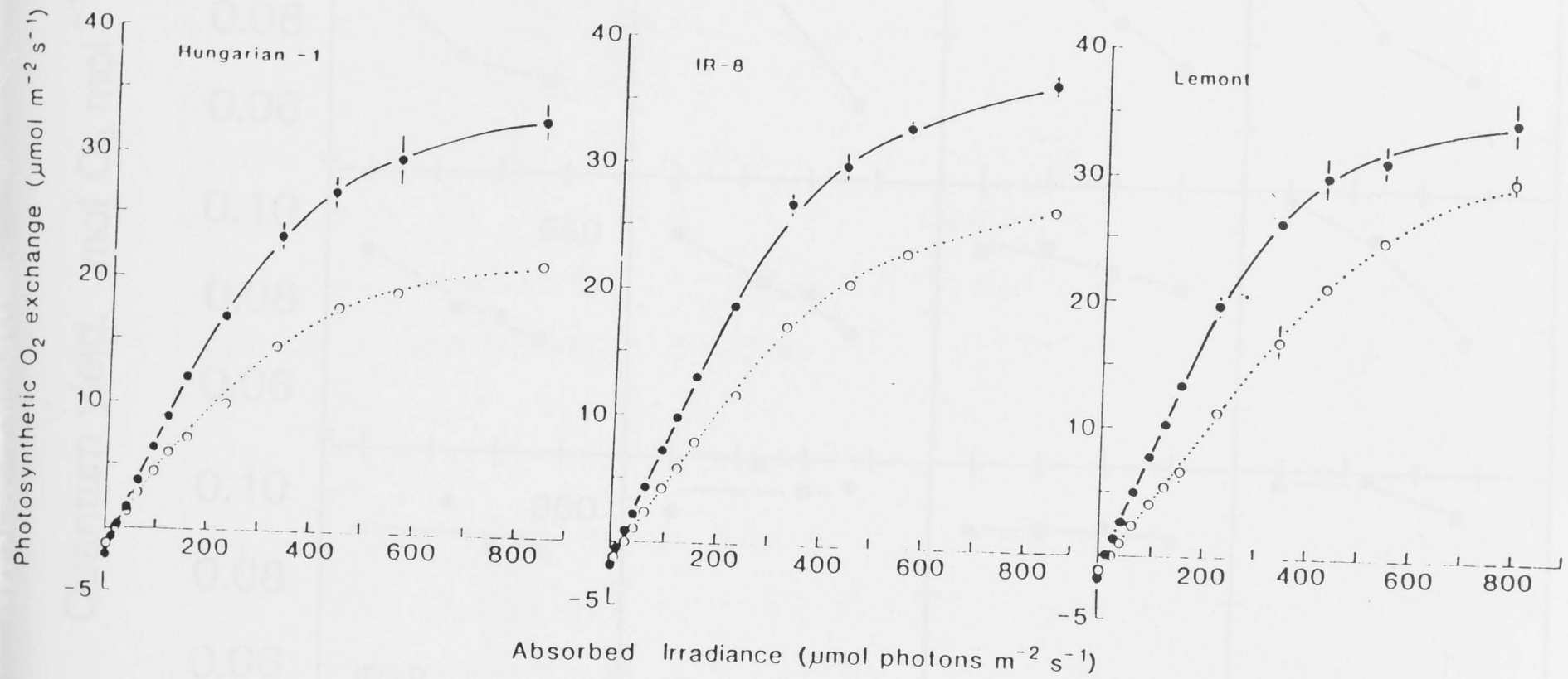

Figure 3. 2 Light response curves of photosynthesis in leaves of rice cultivars before (●) and after (○) exposure of attached leaves to bright light (9 h at 1450 μmol photons m^{-2} s^{-1}) in air at 25 °C. Plants were grown in controlled environment chambers.

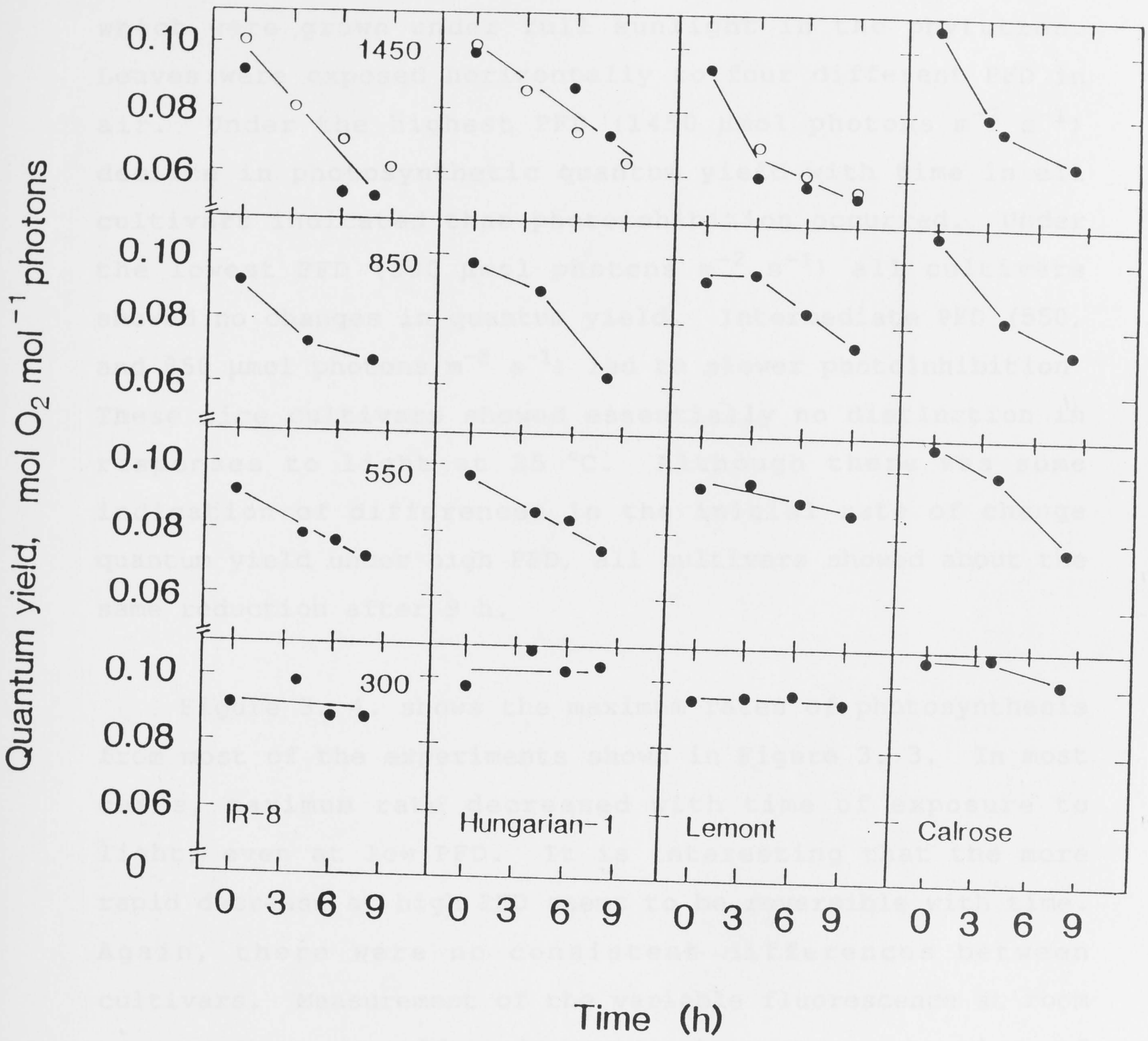

Figure 3. 3 Changes of quantum yield of the flag leaves of four rice cultivars against time. The attached leaves were exposured horizontally to four different PFD in air.

which were grown under full sunlight in the phytotron. Leaves were exposed horizontally to four different PFD in air. Under the highest PFD (1450 μmol photons m^{-2} s^{-1}) decline in photosynthetic quantum yield with time in all cultivars indicated that photoinhibition occurred. Under the lowest PFD (300 μmol photons m^{-2} s^{-1}) all cultivars showed no changes in quantum yield. Intermediate PFD (550, and 850 μmol photons m^{-2} s^{-1}) led to slower photoinhibition. These rice cultivars showed essentially no distinction in responses to light at 25 °C. Although there was some indication of differences in the initial rate of change quantum yield under high PFD, all cultivars showed about the same reduction after 9 h.

Figure 3. 4. shows the maximum rates of photosynthesis from most of the experiments shown in Figure 3. 3. In most cases, maximum rate decreased with time of exposure to light, even at low PFD. It is interesting that the more rapid decrease at high PFD seems to be reversible with time. Again, there were no consistent differences between cultivars. Measurement of the variable fluorescence at room temperature in all cultivars showed a reduction of fluorescence which was time and PFD dependent (Figure 3. 5). Unlike the changes in quantum yield, fluorescence was significantly inhibited even at low PFD (compare 300 μmol photons m^{-2} s^{-1} treatments in Figures 3. 3 and 3. 5). In these experiments there was a tendency for recovery of fluorescence with time, but again, there were no consistent differences between cultivars.

It was difficult to control the temperature of attached leaves to 10 °C during photoinhibition in air, so detached

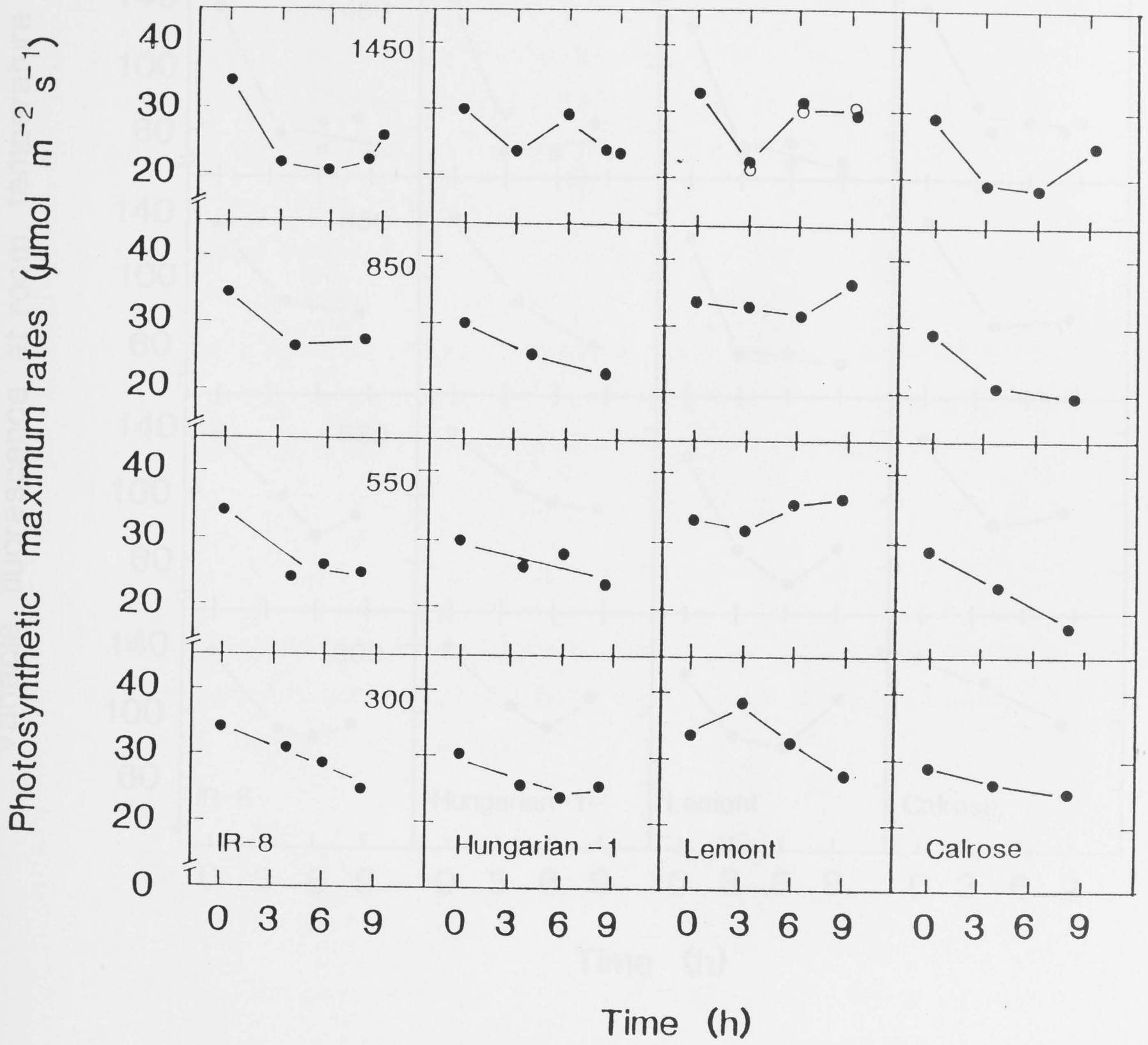

Figure 3. 4 Changes of maximum photosynthesis rate of the flag leaves of four cultivars against time. The attached flag leaves were exposed horizontally to four different PFD in air.

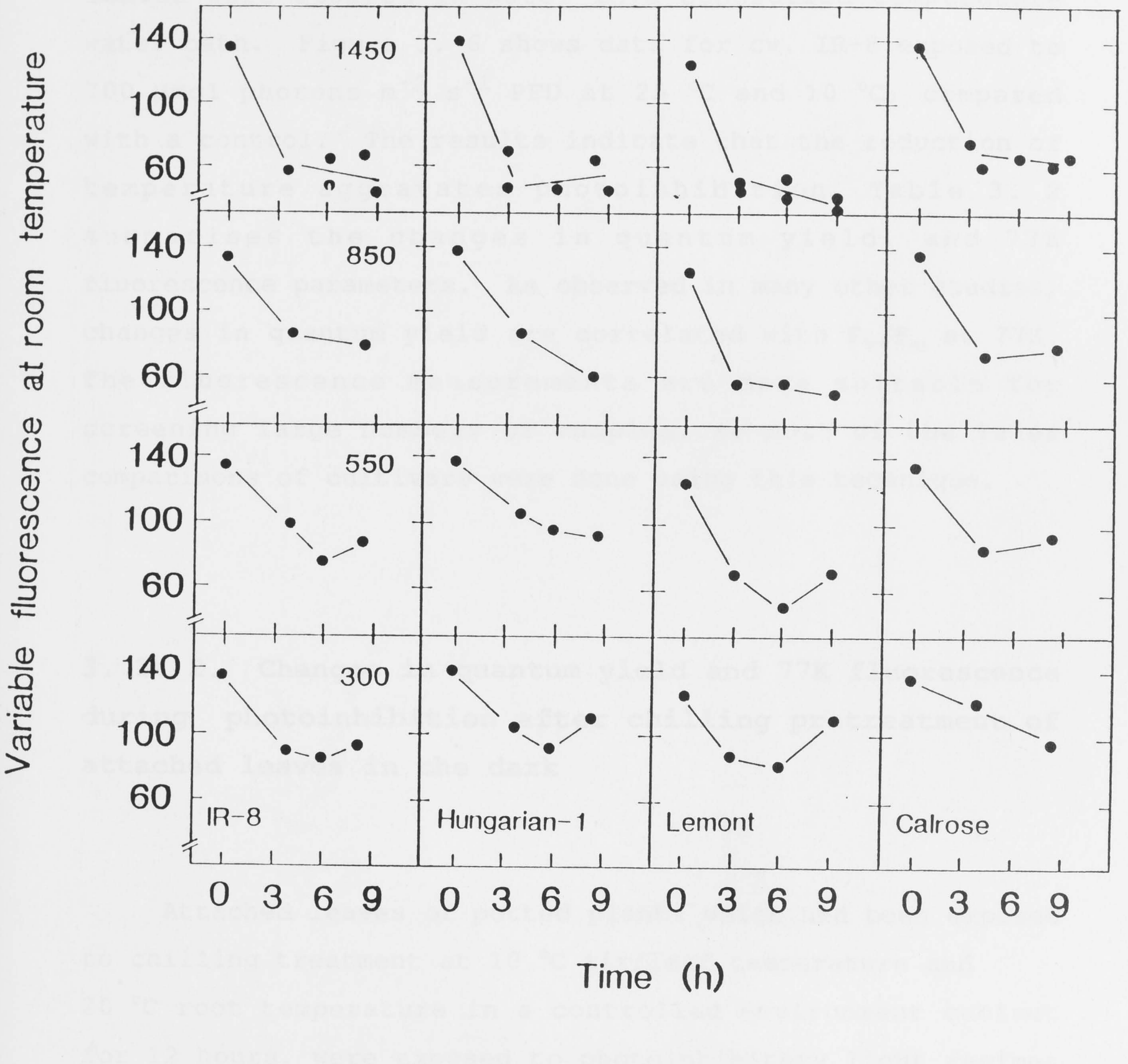

Figure 3. 5 Changes of the variable fluorescence (room temperature) of the flag leaves of four rice cultivars against time. The attached flag leaves were exposed horizontally to four different PFD in air.

leaves were floated in water in a controlled temperature water bath. Figure 3. 6 shows data for cv. IR-8 exposed to 700 μmol photons m^{-2} s^{-1} PFD at 25 °C and 10 °C, compared with a control. The results indicate that the reduction of temperature aggravates photoinhibition. Table 3. 2 summarises the changes in quantum yield, and 77K fluorescence parameters. As observed in many other studies, changes in quantum yield are correlated with F_v/F_m at 77K. The fluorescence measurements are more suitable for screening large numbers of samples, so most of the later comparisons of cultivars were done using this technique.

3. 3. 2. Changes in quantum yield and 77K fluorescence during photoinhibition after chilling pretreatment of attached leaves in the dark

Attached leaves of potted plants which had been exposed to chilling treatment at 10 °C air/leaf temperature and 20 °C root temperature in a controlled environment cabinet for 12 hours, were exposed to photoinhibitory light regimes (700 μmol photons m^{-2} s^{-1}, at 25 °C). Leaf segments were taken for quantum yield measurements, and small discs were punched from adjacent portions of the same leaves at the same time for the 77K fluorescence measurements. The results of the experiments in five rice cultivars are shown in Table 3. 3. In all cultivars, except cv. Hungarian-1 grown in the CSIRO phytotron, 8 h exposure to 700 μmol photons m^{-2} s^{-1} led to a significant reduction in quantum yield which was accompanied by a proportionally similar

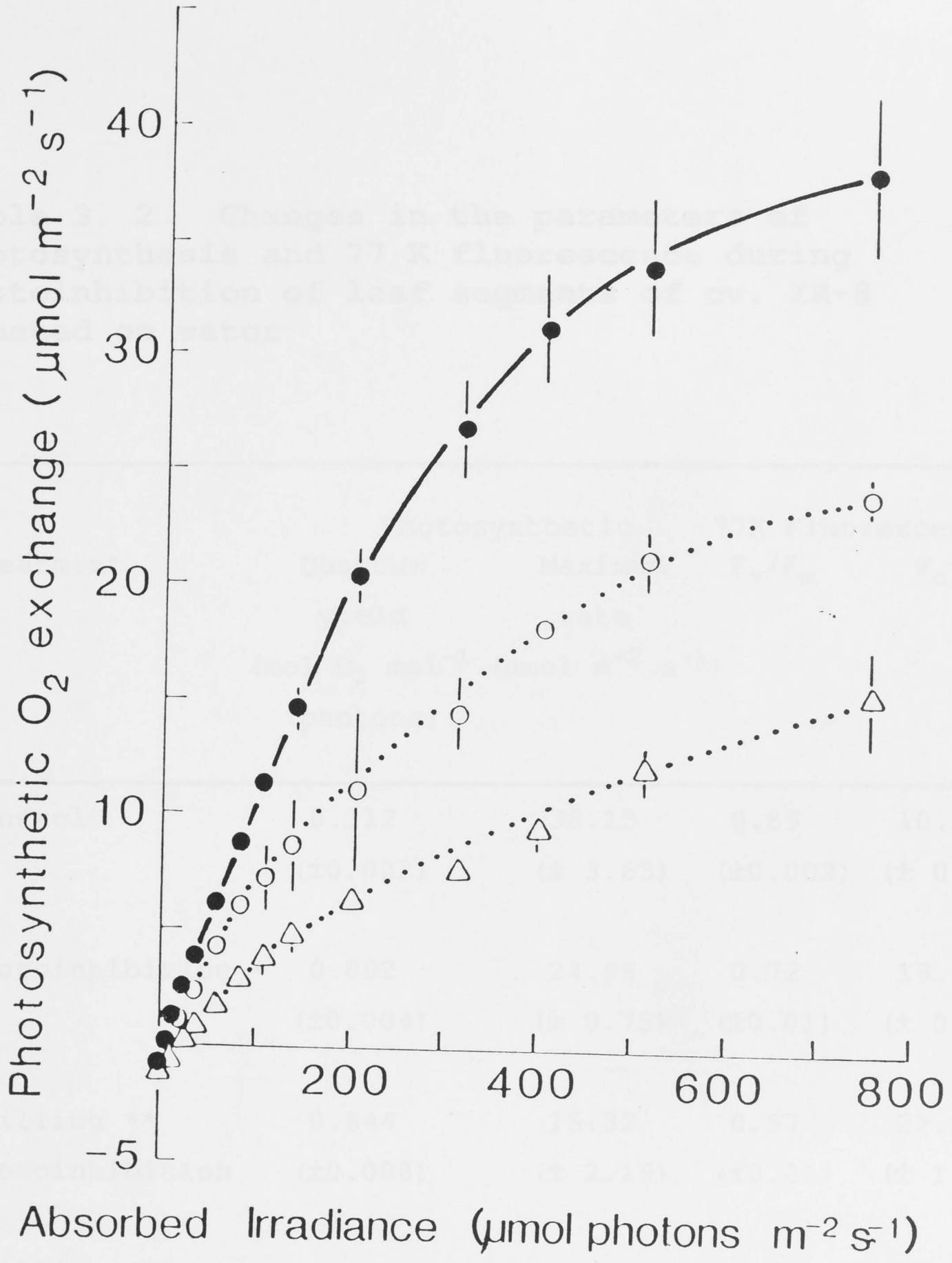

Figure 3. 6 Light response curves for photosynthetic O_2 evolution in detached leaf segments of rice cv. IR-8, incubated on water at 25 °C in the dark (●), in the light (700 µmol photons m^{-2} s^{-1}) at 25 °C (○) or in the light at 10 °C (△).

Table 3. 2. Changes in the parameters of photosynthesis and 77 K fluorescence during photoinhibition of leaf segments of cv. IR-8 floated on water

Treatment	Photosynthetic Quantum yield (mol O_2 mol^{-1} photons)	Maximum rate (μmol m^{-2} s^{-1})	77K Fluorescence F_v/F_m	F_o
Control	0.112 (±0.002)	38.15 (± 3.65)	0.85 (±0.003)	10.71 (± 0.19)
Photoinhibition *	0.092 (±0.004)	24.08 (± 0.75)	0.72 (±0.01)	19.72 (± 0.79)
Chilling ** photoinhibition	0.044 (±0.008)	15.32 (± 2.19)	0.57 (±0.01)	22.20 (± 1.00)
Chilling *** in the dark	-	-	0.87 (±0.01)	16.00 (± 0.11)

* Leaves exposed to 700 µmol photons m^{-2} s^{-1} for 8 h at 25 °C.

** Leaves exposed to 700 µmol photons m^{-2} s^{-1} for 8 h at 10 °C.

*** 10 °C in the dark for 8 h.

Table 3. 3 Effects of chilling on susceptibility to photoinhibition in different varieties of *Oryza* .

Variety and growth conditions	Parameter	Control[1]	Photoinhibition[2]	Chilling[3] alone	Chilling +[4] Photoinhibition
Hungarian 1, Phytotron	QY[5]	99 ± 1	98 ± 1	98	87 ± 3
	F_o[6]	16.6 ± 1.1	25.3 ± 2.5	19.3 ± 0.5	32.9 ± 4.2
	F_v/F_m[7]	0.85 ± 0.01	0.72 ± 0.02	0.85	0.66 ± 0.03
ANU chamber	QY	93 ± 6	79 ± 4	92 ± 4	73 ± 7
	F_o	15.6 ± 1.2	30.6 ± 6.0	15.2 ± 1.7	24.90 ± 1.5
	F_v/F_m	0.85 ± 0.01	0.68 ± 0.03	0.85 ± 0.03	0.71 ± 0.03
Er Bai Ai, Phytotron	QY	92 ± 4	67	91	67
	F_o	16.3 ± 1.8	32.8 ± 4.1	14.4 ± 2.0	23.4 ± 3.8
	F_v/F_m	0.86 ± 0.02	0.63 ± 0.02	0.86 ± 0.02	0.77 ± 0.04
Gui Chao 2, Phytotron	QY	92 ± 5	77 ± 8	88 ± 15	79 ± 11
	F_o	15.2 ± 2.5	28.9 ± 5.1	15.1 ± 3.5	26.2 ± 4.6
	F_v/F_m	0.86 ± 0.01	0.65 ± 0.03	0.84 ± 0.2	0.69 ± 0.03
Lemont, Phytotron	QY	88 ± 4	68	75	70
	F_o	16.6 ± 1.6	28.6 ± 3.8	16.2 ± 1.4	23.5 ± 3.7
	F_v/F_m	0.86 ± 0.02	0.67 ± 0.05	0.85 ± 0.01	0.70 ± 0.05
IR-8, Phytotron	QY	91 ± 1	76 ± 4	80 ± 4	75 ± 2
	F_o	16.8 ± 1.6	27.1 ± 5.4	16.2 ± 1.3	20.6 ± 0.6
	F_v/F_m	0.84 ± 0.02	0.69 ± 0.07	0.84 ± 0.02	0.76 ± 0.04
ANU chamber	QY	90 ± 3	79	83 ± 12	63 ± 13
	F_o	14.0 ± 0.6	30.8 ± 1.9	15.5 ± 0.5	28.9 ± 3.0
	F_v/F_m	0.87 ± 0.01	0.69 ± 0.07	0.84 ± 0.03	0.63 ± 0.06

(1) Mean of attached leaves on plants from growth environment, morning and evening

(2) Leaves of attached plants exposed to 700 µmol photons m^{-2} s^{-1} at 25°C for 8 h

(3) Leaves of attached plants chilled in the dark at 10°C for 12 h (roots at 20°C)

(4) Leaves of attached plants chilled in the dark at 10°C for 12 h (roots at 20°C) then exposed to 700 µmol photons m^{-2} s^{-1} at 25°C for 8 h

(5) Mean of 2 to 6 experiments, mmol O_2 mol^{-1} absorbed photons

(6) Arbitary units, mean of 4 to 8 measurement in each experiment and of 2 to 6 experiments except for cv. Lemont

(7) Mean of 4 to 8 measurements in each experiment and of 2 to 6 experiments except for cv. Lemont.

decrease in F_v/F_m of 77K fluorescence, and an increase in F_o. Chilling in the dark for 12 h with 10 °C had a scarcely detectable effect on the quantum yield in cv. Hungarian-1, Er Bai Ai, or Gui Chao-2, and only a small effect on cv. IR-8 (both grown in the phytotron and cabinet) and cv. Lemont, and no effect on 77K fluorescence. When chilling was followed by

8 h in 700 μmol photons m^{-2} s^{-1} at 25 °C, there was no additional effect of chilling on the extent of photoinhibition, except in cv. IR-8 from the ANU growth chamber.

The increase in F_o observed in these experiments was closely correlated with the decrease in F_v/F_m, as is shown in Figure 3. 7. However, it was not the main cause of the decline in F_v/F_m, which reflects principally a decrease in F_m. In general, the data show that one night of chilling at 10 °C does not predispose rice leaves to subsequent further photoinhibition in bright light. If these criteria are to be employed to screen differences between varieties, a better system for treatment and measurement is required. To this end, we compared the responses of lamina segment from detached leaves which were floated on water under controlled light and temperature conditions.

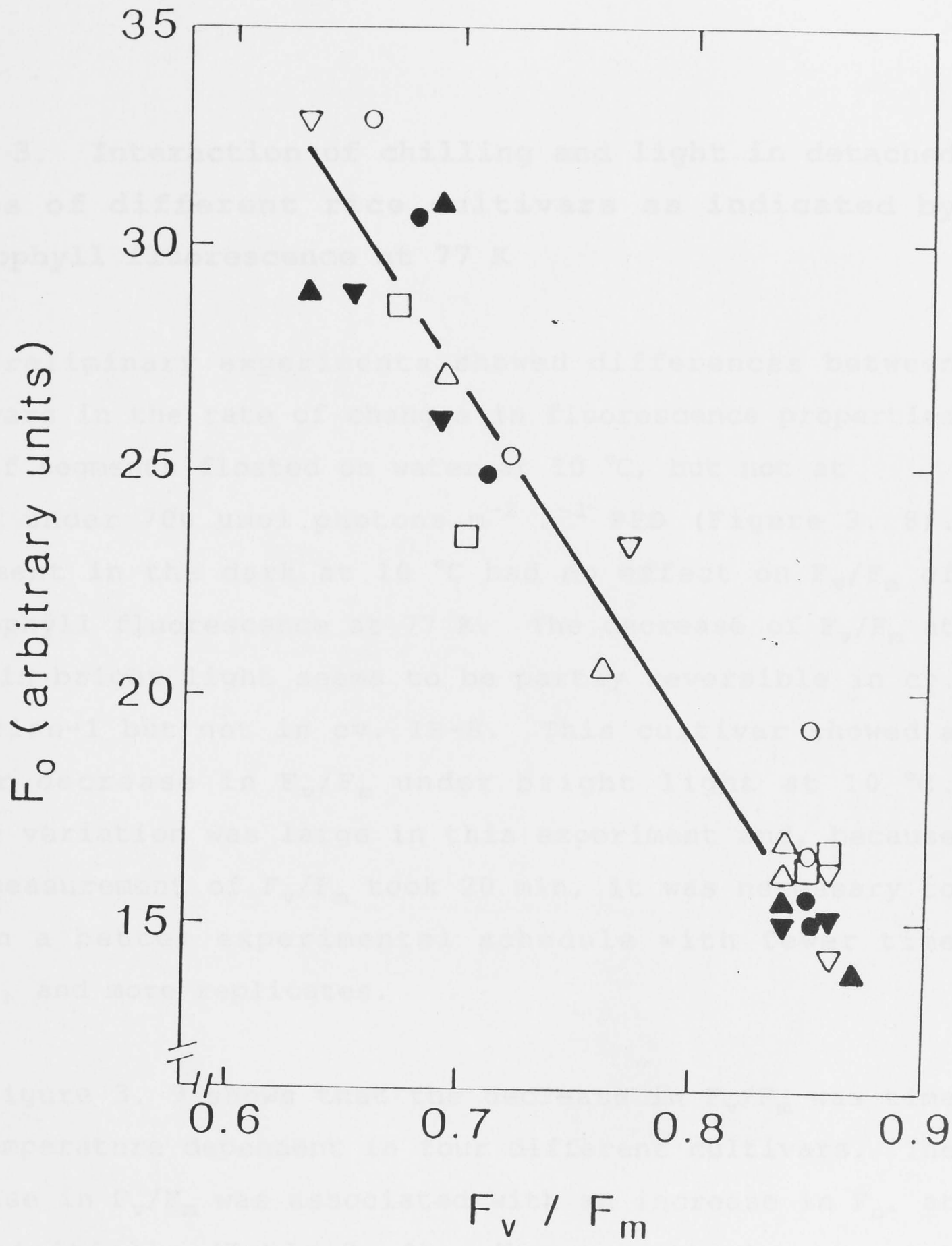

Figure 3. 7 Correlation (drawn by eye) between F_o and F_v/F_m for 77 K chlorophyll fluorescence during photoinhibition of different rice cultivars in air. Data drawn from Table 3. 3; different symbols refer to different cultivars. Cultivars grown in controlled environment cabinets (closed symbols) are distinguished from those grown in the phytotron (open symbols) as follows: Hungarian-1 (●, ○); IR-8 (▲, △); Lemont (❑); Er Bai Ai (▽); and Gui Chao-2 (▼).

3. 3. 3. Interaction of chilling and light in detached leaves of different rice cultivars as indicated by chlorophyll fluorescence at 77 K

Preliminary experiments showed differences between cultivars in the rate of changes in fluorescence properties of leaf segments floated on water at 10 °C, but not at 25 °C, under 700 μmol photons m^{-2} s^{-1} PFD (Figure 3. 8). Treatment in the dark at 10 °C had no effect on F_v/F_m of chlorophyll fluorescence at 77 K. The decrease of F_v/F_m at 25 °C in bright light seems to be partly reversible in cv. Hungarian-1 but not in cv. IR-8. This cultivar showed a larger decrease in F_v/F_m under bright light at 10 °C. Sample variation was large in this experiment and, because each measurement of F_v/F_m took 20 min, it was necessary to design a better experimental schedule with fewer time points, and more replicates.

Figure 3. 9 shows that the decrease in F_v/F_m was time and temperature dependent in four different cultivars. The decrease in F_v/F_m was associated with an increase in F_o, at least initially (Table 3. 4). However, the measurement errors in F_o are large, compared with F_v/F_m, and so the parameter F_v/F_m was used in most experiments. In another experiment, changes in F_v/F_m were examined over prolonged time intervals (up to 72 h) at different temperatures, (10, 15, and 25 °C), during 12 h day (light, 700 μmol photons m^{-2} s^{-1})/12 hours night (dark)

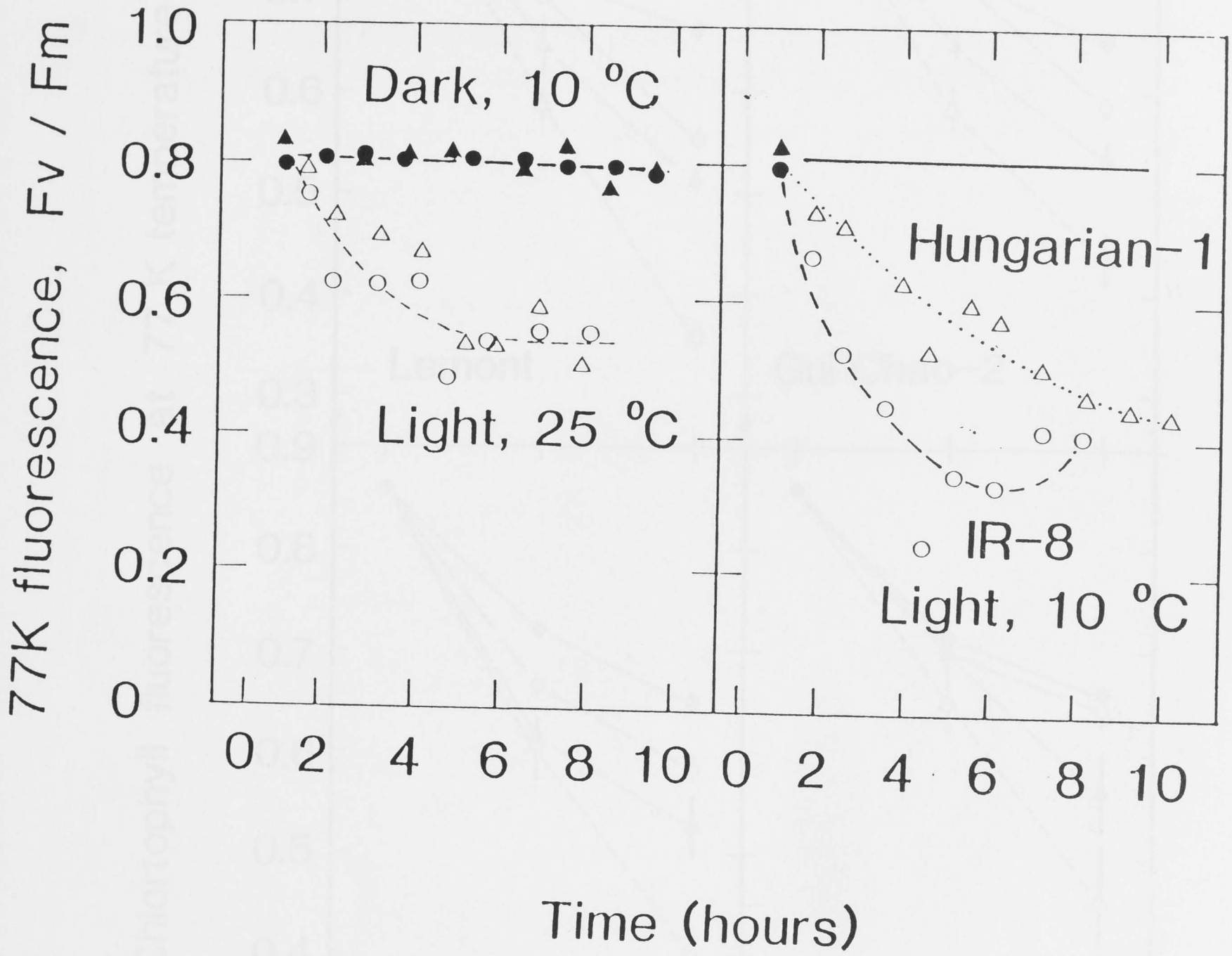

Figure 3. 8 Changes in F_v/F_m of 77K fluorescence in rice cultivars kept at 10 °C in the dark (▲, ●) or in the light (Δ, ○) at 25 or 10 °C.

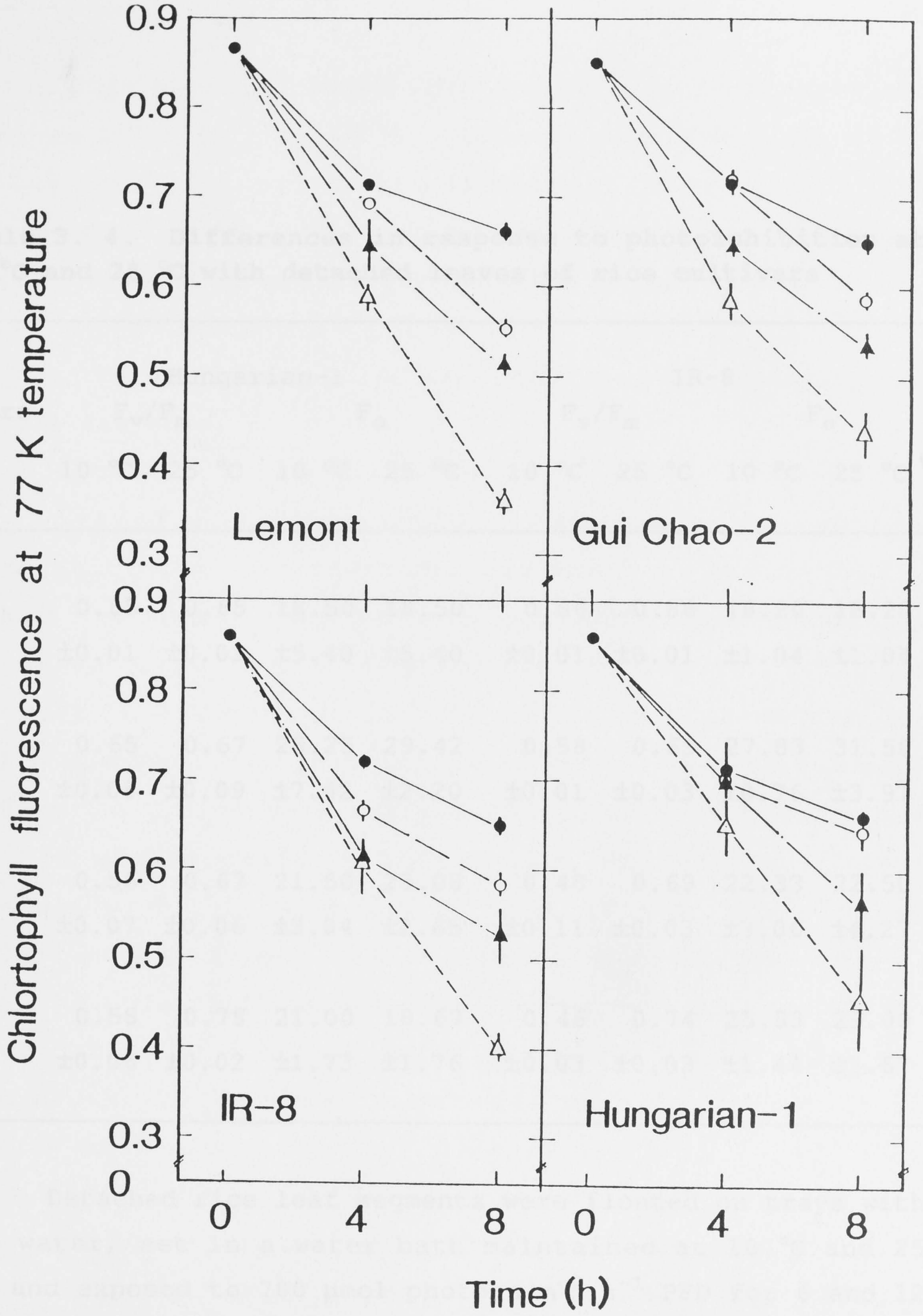

Figure 3. 9 Time dependent decrease of F_v/F_m at four different temperatures in four different cultivars. Leaf segments were floated on water at 25 °C (●), 18 °C (○), 15 °C (▲) and 10 °C (△) under 700 μmol photons m^{-2} s^{-1}.

Table 3. 4. Differences in response to photoinhibition at 10 °C and 25 °C with detached leaves of rice cultivars

Hours	Hungarian-1				IR-8			
	F_v/F_m		F_o		F_v/F_m		F_o	
	10 °C	25 °C	10 °C	25 °C	10 °C	25 °C	10 °C	25 °C
00	0.85 ±0.01	0.85 ±0.01	18.50 ±5.40	18.50 ±5.40	0.86 ±0.01	0.86 ±0.01	18.20 ±1.04	18.20 ±1.04
06	0.65 ±0.08	0.67 ±0.09	23.25 ±7.42	29.42 ±2.20	0.58 ±0.01	0.63 ±0.03	27.83 ±0.76	31.50 ±3.97
12	0.56 ±0.07	0.67 ±0.06	21.50 ±3.04	23.00 ±2.65	0.48 ±0.11	0.69 ±0.03	22.33 ±3.06	22.50 ±4.27
24	0.58 ±0.06	0.78 ±0.02	21.00 ±1.73	18.67 ±1.76	0.46 ±0.03	0.74 ±0.03	25.83 ±1.44	29.00 ±3.67

Detached rice leaf segments were floated on trays with tap water, set in a water bath maintained at 10 °C and 25 °C, and exposed to 700 µmol photons m^{-2} s^{-1} PFD for 6 and 12 h, and maintained in the dark at same temperature conditions for 12 h recovery period.

cycles. In some cultivars (cv. Lemont) leaf yellowing was observed after 72 h at 10 °C. However, over the first 48 h, two cycles of temperature dependent decrease in 77K fluorescence in the light showed the temperature dependency of photoinhibition and recovery in the dark (Figure 3. 10). These experiments show the need to separate the light-dependent, inhibitory phase, and dark recovery phase in the overall response.

Some of the interactions between temperature, photoinhibition, and recovery, are shown in Figure 3. 11. As observed above, there was not much difference between cv. Hungarian-1 and IR-8 during photoinhibition at 25 °C, and recovery in the dark at 25 °C was also similar (Figure 3. 11a). However, when photoinhibited leaves were kept in the dark at 10 °C, recovery was reduced in both cultivars, and cv. IR-8 showed greater reduction of F_v/F_m during subsequent exposure to bright light at 10 °C (Figure 3. 11b). These results were confirmed when photoinhibition was done at 10 °C. Figure 3. 11c shows that low temperature exaggerates the decrease in F_v/F_m, especially in cv. IR-8. However, recovery at 25 °C in the dark after photoinhibition at 10 °C, was much greater in cv. Hungarian-1 than in cv. IR-8 (Figure 3. 11d). It was interesting that subsequent exposure to bright light at 25 °C did not lead to more photoinhibition, or prevent further recovery. Further experiments are needed to understand these interactions, but these experiments show that it is possible to design a simple experimental schedule to screen for differences between cultivars.

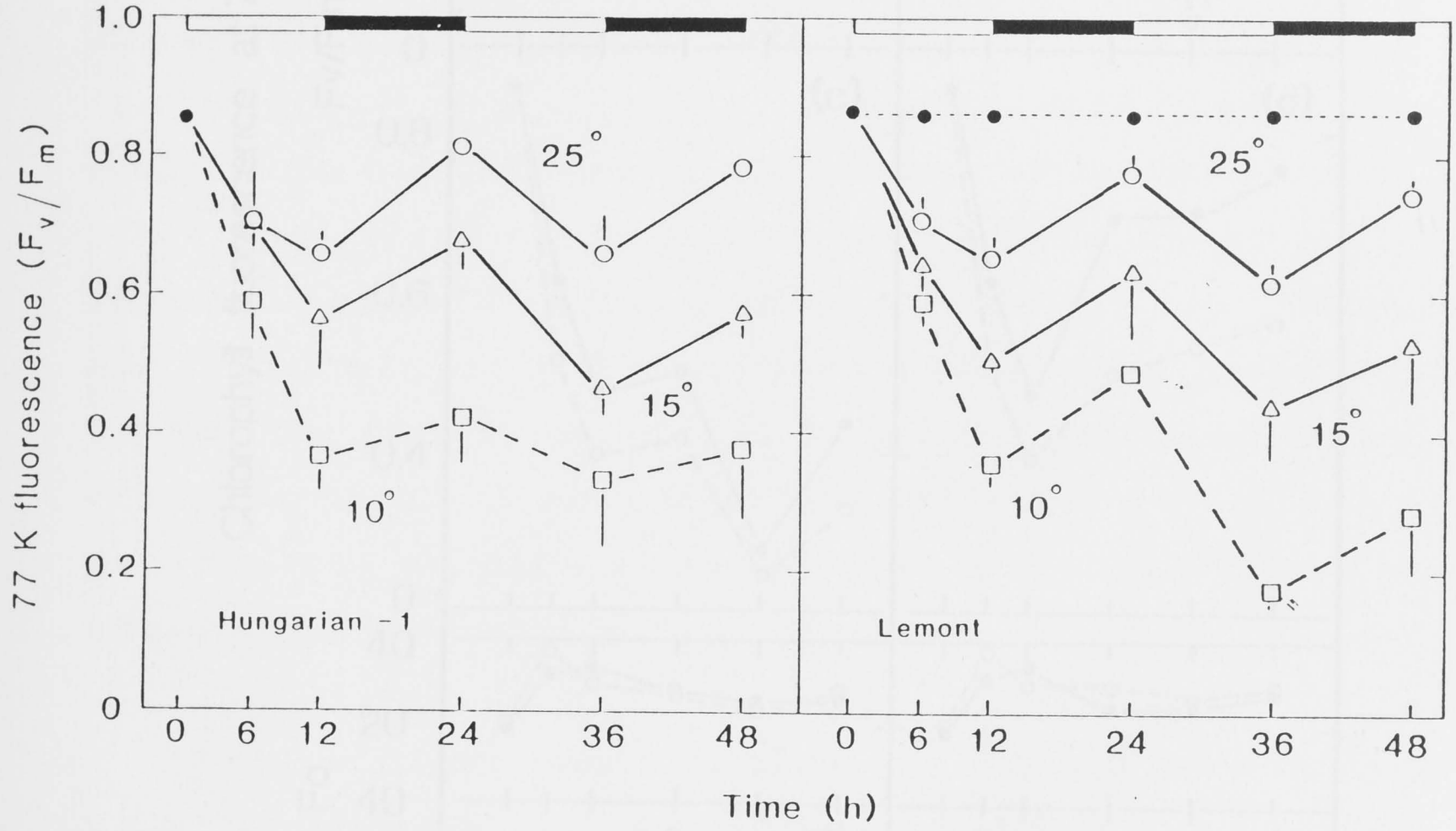

Figure 3. 10 Time course of changes in F_v/F_m of 77K chlorophyll fluorescence in leaf segments of rice cultivars incubated on water in the light (700 µmol photons m^{-2} s^{-1}) (open symbols) or dark (●) at different temperatures; (○) 25 °C; (△) 15 °C; (□) 10 °C.

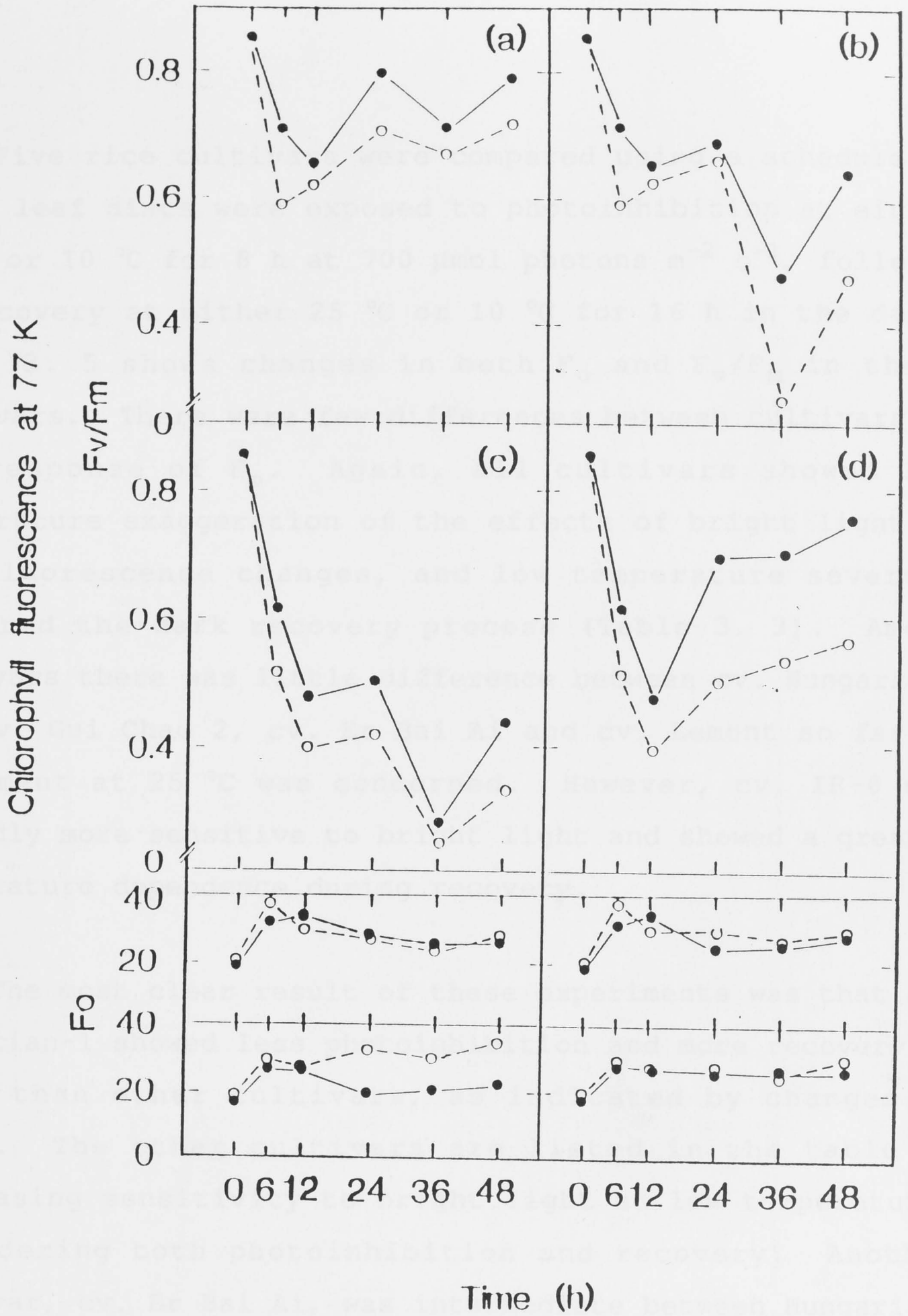

Figure 3. 11 Interaction between temperature, photoinhibition (as indicated by F_v/F_m) and recovery in cv. Hungarian-1 (●) and cv. IR-8 (○). (a) photoinhibition at 25 °C and recovery in the dark at 25 °C; (b) photoinhibition at 25 °C and recovery in the dark at 10 °C; (c) photoinhibition at 10 °C and recovery in the dark at 10 °C; (d) photoinhibition at 10 °C and recovery in the dark at 25 °C.

Five rice cultivars were compared using a schedule in which leaf discs were exposed to photoinhibition at either 25 °C or 10 °C for 8 h at 700 μmol photons m^{-2} s^{-1}, followed by recovery at either 25 °C or 10 °C for 16 h in the dark. Table 3. 5 shows changes in both F_o and F_v/F_m in these cultivars. There were few differences between cultivars in the response of F_o. Again, all cultivars showed low temperature exaggeration of the effects of bright light on 77K fluorescence changes, and low temperature severely impaired the dark recovery process (Table 3. 3). Among cultivars there was little difference between cv. Hungarian-1, cv. Gui Chao 2, cv. Er Bai Ai and cv. Lemont so far as treatment at 25 °C was concerned. However, cv, IR-8 was markedly more sensitive to bright light and showed a greater temperature dependence during recovery.

The most clear result of these experiments was that cv. Hungarian-1 showed less photoinhibition and more recovery at 10 °C than other cultivars, as indicated by changes in F_v/F_m. The other cultivars are listed in the table in increasing sensitivity to bright light at low temperature, considering both photoinhibition and recovery. Another cultivar, cv. Er Bai Ai, was intermediate between Hungarian-1 and the others, especially so far as recovery in the dark at 25 °C was concerned. The three remaining cultivars, cv. Gui Chao-2, Lemont and IR-8 were markedly more sensitive than cv. Hungarian-1. As observed previously (Figure 3. 7), the changes in F_o were closely correlated with changes in F_v/F_m in all cultivars. However, when bright light was accompanied by low temperature, a larger decrease in F_v/F_m was observed for a given increase in F_o (Figure 3. 12).

Table 3. 5 Effect of temperature on 77K fluorescence parameters during photoinhibition and recovery in different varieties of *Oryza* (n = 6).

Variety	Control	Photoinhibition[1] 25°	Recovery[2] 25°	Recovery[2] 10°	Photoinhibition[3] 10°	Recovery[2] 25°	Recovery[2] 10°
Hungarian 1							
F_o	20 ± 3	32 ± 3	23 ± 3	26 ± 3	31 ± 3	25 ± 4	27 ± 2
F_v/F_m	0.86 ± 0.02	0.68 ± 0.03	0.83 ± 0.02	0.77 ± 0.01	0.61 ± 0.03	0.78 ± 0.03	0.67 ± 0.02
Er Bai Ai							
F_o	19 ± 2	35 ± 6	23 ± 3	27 ± 2	33 ± 3	21 ± 0	31 ± 2
F_v/F_m	0.85 ± 0.02	0.66 ± 0.08	0.81 ± 0.03	0.75 ± 0.04	0.51 ± 0.06	0.76 ± 0.02	0.57 ± 0.02
Gui Chao 2							
F_o	19 ± 2	31 ± 4	23 ± 3	26 ± 0	32 ± 2	27 ± 2	31 ± 4
F_v/F_m	0.86 ± 0.01	0.69 ± 0.06	0.83 ± 0.04	0.75 ± 0.01	0.50 ± 0.04	0.70 ± 0.01	0.54 ± 0.02
Lemont							
F_o	20 ± 2	32 ± 2	23 ± 1	29 ± 3	35 ± 4	33 ± 6	39 ± 3
F_v/F_m	0.86 ± 0.01	0.69 ± 0.02	0.81 ± 0.00	0.73 ± 0.02	0.51 ± 0.06	0.69 ± 0.10	0.49 ± 0.05
IR-8							
F_o	20 ± 2	43 ± 7	28 ± 2	37 ± 6	32 ± 8	26 ± 2	32 ± 1
F_v/F_m	0.83 ± 0.03	0.58 ± 0.06	0.79 ± 0.00	0.68 ± 0.03	0.49 ± 0.06	0.71 ± 0.04	0.50 ± 0.05

[1] Leaf segments exposed to 700 µmol photons m^{-2} s^{-1} for 8 h at 25°

[2] Leaf segments kept in the dark for 16 h at 25° or 10° after photoinhibition

[3] Leaf segments exposed to 700 µmol photons m^{-2} s^{-1} for 8 h at 10°

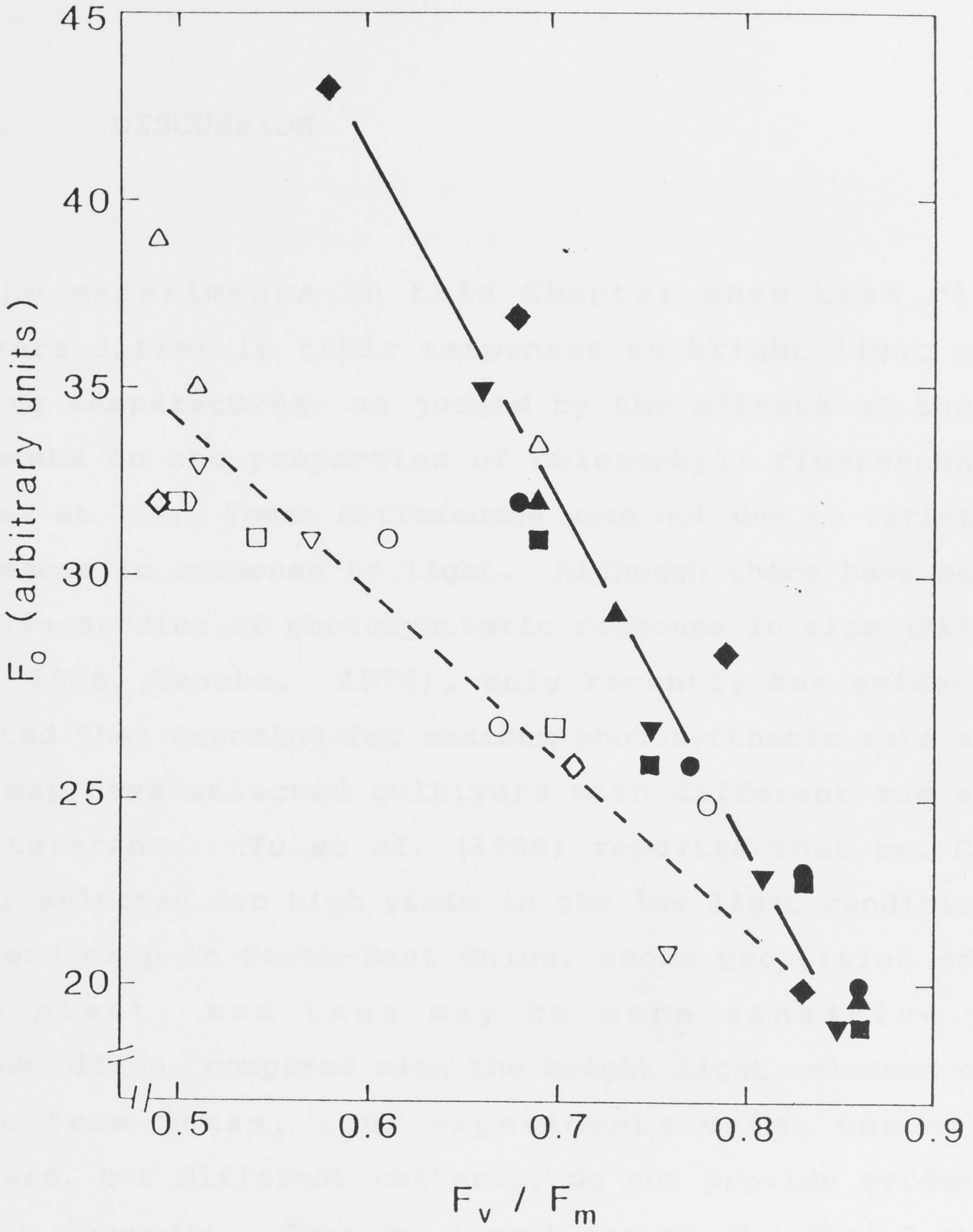

Figure 3. 12 Correlation (drawn by eye) between F_o and F_v/F_m for 77 K chlorophyll fluorescence in leaves of rice cultivars following photoinhibition at 25 °C (closed symbols) or 10 °C (open symbols). Data drawn from Table 3. 5; different symbols refer to different cultivars as follows: Hungarian-1 (●, ○); IR-8 (◆, ◇); Lemont (▲, △); Er Bai Ai (▼, ▽); and Gui Chao-2 (■, ❑).

3. 4. DISCUSSION

The experiments in this Chapter show that rice cultivars differ in their responses to bright light and chilling temperatures, as judged by the effects of these treatments on the properties of chlorophyll fluorescence measured at 77K. These differences were not due to varietal differences in response to light. Although there have been extensive studies of photosynthetic response in rice (Akita *et al.* 1968; Tanaka, 1976), only recently has evidence suggested that breeding for maximum photosynthetic rate and yield may have selected cultivars with different sun and shade tolerance. Tu *et al.* (1988) reported that cv. Gui Chao-2, selected for high yield in the low light conditions of second crop in South-East China, shows properties of a shade plant, and thus may be more sensitive to photoinhibition, compared with the bright light selected cv. Lemont from Texas. Our experiments using the same cultivars, but different criteria, do not provide evidence for this viewpoint. Thus cv. Lemont and cv. Gui Chao-2 show similar responses to shading (Figure 3. 1), and do not differ in the response of chlorophyll fluorescence to bright light at 25 °C (Table 3. 3). It may be that differences observed by Tu *et al.* (1988) were the result of the different nitrogen status of the plants (Ferrar and Osmond, 1986). Thus it is concluded that the differences between cultivars which were observed when leaves were exposed to combination of bright light and low temperature are due to

differences in the interaction with low temperature, not to differences in sensitivity to light.

Leaves of all cultivars which were tested were unexpectedly sensitive to photoinhibition at 25 °C. Photoinhibition of leaves was observed when exposed to PFD of 550 μmol photons m^{-2} s^{-1}, or more. It is likely that the nearly vertical arrangement of rice leaves in dense canopies means that leaves develop under PFD which is only a small fraction of the incident PFD (Huang *et al.*, 1986). That is, most rice leaves are probably shade adapted. Under natural conditions, cultivar differences in canopy and leaf arrangement may lead to differences in the extent of adaptation to light in different leaves. This could give differences in the sensitivity to bright light which would complicate interpretation of low temperature effects between cultivars. However, there was no indication of such complications in the present experiments.

Considering the question raised in the introduction, the results of the above experiments show the following:

(1) Treatment of rice leaves at low temperature (10 °C for up to 12 h) in the dark has no effect on quantum yield or 77 K fluorescence. It can be concluded that normal night chilling is unlikely to have an effect on the water splitting reactions of PS II, of the type described by Smillie and Nott (1979). The above results are also consistent with the relative insensitivity of rice leaves to dark chilling described recently by Smillie *et al.* (1988), and Terashima *et al.* (1989).

(2) Dark chilling of rice leaves does not make them more sensitive to photoinhibition in bright light at 25 °C during the next day, contrary to some preliminary indications that this was so (Osmond *et al.*, 1987). There was no evidence for cultivar differences in these experiments.

(3) In rice, as in many other plants, chilling exaggerates photoinhibition. Low temperature increases the effects of bright light on the depression of quantum yield and F_v/F_m of 77 K fluorescence. Cultivars differ in their sensitivity to photoinhibition at low temperature.

(4) Low temperature in the dark reduces the rate of recovery from photoinhibition, as indicated by 77 K fluorescence parameters. Cultivars may differ in the temperature sensitivity of recovery processes.

The last two points should be discussed in more detail. The reasons for increased photoinhibition at low temperature are not clear. Osmond (1981) proposed that photoinhibition would be increased by any environmental factor which reduced the rate of photosynthesis in bright light, simply because the proportion of excitation used in photosynthetic processes was reduced, and the proportion of excitation which had to be used in other ways was increased. This is a very general hypothesis, but because photochemistry is almost independent of temperature, and the biochemistry of photosynthesis decreases many times with a 10 °C decrease in temperature (high Q_{10}), it is not difficult to imagine that over excitation of reaction centres occurs in bright light at low temperature. It follows that the ability of Japonica-

rice to maintain high photosynthetic rate at low temperature, compared with Indica-rice (see Figure 1. 2) may be important, and could explain the differences between cv. Hungarian-1 and IR-8.

In general, the results of experiments reported here are similar to those of Powles *et al.* (1983), Ögren and Öquist (1984 a,b), Vgren *et al.* 1984, and Greer *et al.* (1986, 1988) in that exposure to bright light at low temperature leads to an increase in F_o and a decrease in F_v/F_m for chlorophyll fluorescence at 77K temperature, and also to a decrease in quantum yield. Rice leaves exposed to bright light at 10 °C, F_o did not increase above that already evident in bright light at 25 °C. However, the extent of change in F_v/F_m for a given change in F_o was much greater when rice leaves were exposed to bright light at low temperature (Figure 3. 12). In the experiments reported here, the increase of F_o was followed by a decline as F_v/F_m decreased. The interpretation of 77K fluorescence data, developed from the models of Butler (1978), by Ögren and Vquist, (1984 b), Björkman (1987 a,b) and Krause (1988), suggests that the increase in F_o indicates reduced efficiency of excitation trapping and probable damage to the PS II reaction centre (Cleland *et al.*, 1986; Chow *et al.*, 1989).

All of the changes in fluorescence parameters observed following photoinhibition at 25 or 10 °C were only slowly reversible in the dark, which is also consistent with this conclusion. In other experiments with spinach, the decrease in F_v/F_m following photoinhibition at 10 °C was partly reversible in weak light at 25 °C (Chow *et al.* 1989;

Somersalo and Krause, 1989). Chow *et al.* (1989) gave direct evidence for PS II reaction centre damage in these experiments, and it seems that similar effects could occur in rice.

The interpretation of differences in the extent of change in F_v/F_m is complex. Current models seek to partition the reduction in the variable portion of fluorescence in terms of two processes; those which accompany impaired primary photochemistry of PS II (associated with the increase in F_v/F_m discussed above) and those associated with an increase in non-photochemical quenching, i.e. with an increase in harmless dissipation of excitation as heat (Örgen and Öquist 1984 b; Björkman, 1987a,b ; Demmig and Björkman, 1987; Krause 1988). If the reduction in F_v/F_m in these experiments is primarily due to the former, as Björkman (1987 b) concluded from analyses of the data of Örgen and Öquist (1984 b), obtained with *Lemna* exposed to high irradiance at low temperature, then the rice cultivars may differ in the extent of damage to PS II reaction centres judged by this criterion. Thus, cultivar Hungarian-1 is less susceptible to photoinhibitory damage to PSII than other cultivars. Proof of this interpretation depends on more detailed evaluation of PS II reaction centre structure and function as outlined by Chow *et al.* (1989).

On the other hand, if the reduction in F_v/F_m in these experiments with rice is primarily due to non-photochemical quenching, then the cultivars may differ in their capacity for dissipation of excitation as heat, with cultivars from south-East Asia having a higher capacity than those from Eastern Europe and the USA. Recent experiments which

implicate the xanthophyll cycle, which determines the ratio of violaxanthin to zeaxanthin and controls the loss of excitation as heat (Demmig *et al.*, 1987), should be undertaken with the rice cultivars used here.

The rice cultivars differ in the extent of changes in the F_v/F_m with some, such as cv. Hungarian-1, much less sensitive than others, such as Gui Chao-2 (Table 3. 5). Furthermore, the cultivars which showed larger decrease in the F_v/F_m and in quantum yield following treatment in bright light at low temperature showed slower recovery in F_v/F_m when returned to 25 °C in the dark (Figure 3. 11; Table 3. 5). So that it must be concluded that in rice plants, as in other species, that the extent of this damage to PS II reaction centres is a balance between onset of photoinhibition and repair (Greer *et al.*, 1986; Greer and Laing, 1988 a,b; Greer *et al.* 1988).

Even though it is not yet possible to identify the mechanisms which underlie the different responses of light dependent changes in chlorophyll fluorescence and quantum yield in rice cultivars, it can be asked whether these measurements can be used to select low temperature tolerant cultivars, and how these measurements relate to the processes of yield reduction under natural conditions. Our experiments do indicate the potential value of 77K chlorophyll fluorescence and quantum yield as a screening procedure. Protocols similar to those used in Table 3. 5 clearly identify different sensitivity to low temperature in the light, and differences in recovery from these treatments. There may well be some correlation between low sensitivity to these treatments and other indices of

tolerance to low temperature, such as rate of photosynthesis at low temperature, and phenological characters (earliness, short growth cycle) and early seedling vigour, associated with cold tolerance in cereals. Thus there is merit in further refinement of these and related fluorescence procedures (Smillie *et al.*, 1988) for screening purposes in breeding programs.

CHAPTER 4. PHYSIOLOGICAL ANALYSIS OF VARIETAL DIFFERENCES IN THE EFFECTS OF CHILLING ON THE CANOPY PHOTOSYNTHESIS UNDER SIMULATED "DRY COLD DEW WIND" CONDITIONS

4. 1. INTRODUCTION

The previous Chapter considered the direct effects of low temperature and bright light on primary processes of photosynthesis in different rice cultivars. It was found that low temperature exaggerated photoinhibition more in some cultivars than in others. The treatments used in these laboratory experiments were similar to natural Dry Cold Dew Wind (DCDW) conditions measured in the field.

The first question to be asked in this Chapter is whether such changes in primary processes of photosynthesis really occur in leaves of mature rice plants in canopies under simulated natural DCDW conditions. Related to this question is the problem of whether cultivar differences in sensitivity to photoinhibition at low temperature are responsible for differences in production under field conditions. This is important because, so far, there are few well established cases in which low temperature photoinhibition leads to reduction of crop yield. Baker *et al.* (1989) found decreases in F_v/F_m and quantum yield of mature maize leaves, which followed chilling temperatures in the middle of the growth season in England. The importance of photoinhibition during winter stress in boreal forests is well known (Öquist *et al.* 1987), but the temperatures involved are much lower than those used here.

If photoinhibition is not responsible for the reduction of photosynthesis under natural conditions, then indirect effects of low temperatures must be considered. Because CDW are important factors determining rice yields in South China, it is not surprising that this distinctive form of chilling injury has attracted attention among researchers in mainland China (Yu *et al.*, 1980) as well as in Taiwan Province of China (Lin and Hung, 1979). Most attention has been given to the indirect effects of low temperature and low light on the translocation of assimilates and the grain filling process (Lin and Tarn, 1979; Wang *et al.*, 1980). Lin and Hung (1979) concluded that low light had a larger effect than low temperature. However, Fu *et al.* (1983) found the after effects were less severe when plants were returned to the normal temperature condition with low light.

One of the most common indirect effects of low temperature is the inhibition of photosynthesis by sugar accumulation in leaves. It has long been known that soluble sugar accumulation in leaves has a feedback, inhibitory effect on the rate of photosynthesis (King *et al.*, 1967; Neales and Incoll, 1968; Claussen and Biller, 1977). Correlations based on girdling experiments with leaves of C_3 plants (Azcón-Bieto, 1983) and a C_4 plant (Blechschmidt-Schneider *et al.*, 1989) continue to support this idea.

Although Wang *et al.* (1980) commented on the inhibitory effects of low temperature on labelled assimilate transport from rice leaves, the only study of a possible relationship between sucrose accumulation and reduced photosynthesis in rice is that of Kishitani and Tsunoda (1974). This report,

only discovered after the experiments described in this Chapter were completed, used 3 day low temperature treatments to check effects on photosynthesis of Indica and Japonica-rice cultivars. The greater reduction of photosynthesis in Indica-rice was associated with a larger accumulation of sugar in leaves of these cultivars

Another indirect effect, which may be related the above soluble sugar response, is linked to temperature responses of root function. Low root temperature reduces water uptake and transport to the shoot, leading to water stress. McWilliam *et al.* (1982) described the chilling induced water stress in *Gossypium hirsutum* and *Phaseolus vulgaris* chilled to 5 °C in the light. They proposed that the low temperatures in the light decreased the hydraulic conductivity of roots to water, and slowed stomatal closing responses so that the shoot water status decreased to the extent that leaves wilted. Low temperature effects on root process in rice seedlings have been examined in South Korea (Lee, 1979), but there is little information which is relevant to mature second crop rice. However, it is a common cultivation method in South China to flood fields with flowing river water during CDW events. As shown in Figure 2. 2, root zone temperature decreases rapidly during CDW, so flooding with warmer river water would maintain higher root temperatures. This practical method suggests that root temperature may have an important physiological role, related to translocation or water stress, in protecting against low temperature effects on leaf processes.

In this Chapter, effects of simulated CDW and natural field conditions on primary photochemical reactions of photosynthesis were evaluated and responses of canopy photosynthesis of different rice cultivars are described. Differences between the separate leaf and the canopy responses on the simulated CDW conditions or natural chilling stress event will be described. Further, the possibility of using the techniques for screening chilling-sensitivity in cultivars of rice will be discussed.

4. 2. MATERIALS AND METHODS

Rice cultivars used for experiments reported in this Chapter were grown as described at Section 2. 3. Plants used for detached leaf experiments were grown in the CSIRO phytotron, and those used for canopy photosynthesis measurements were grown in RSBS glasshouses. The developmental stage of plants at the time of experiments and the method of samples collection are as described at Section 3. 2.

A major method of experiment used here was based on the Weiss controlled environment chambers (see section 2. 4. 2) for the canopy photosynthesis measurement under simulated DCDW conditions. When plants reached the heading/flowering stages they were transferred from glasshouse to Weiss chambers and, depending on the cultivar and experiments, 12-24 pots each containing five plants, with up to four tillers per plant, were used to form a canopy of 2-5 m^2

total leaf area. Pots stood in a tray 75 cm x 70 cm x 15 cm with 10 cm deep nutrient solution (see Table 2. 1) which was either heated to constant 20 °C or allowed to vary with changes in temperature of the aerial environment. The level of solution was maintained by addition of distilled water every day. Temperatures used to construct the daily temperature curves in the chamber were those from the field microclimate records data as described at Section 2. 2 (10 h, 30°C day/20 °C night, for the control experiments; and 10 h, 20 °C day/10 °C night, for the chilling treatment). Daily changes in temperature corresponded with a programmed change in PFD, which was recorded at the top of the canopy. Root temperature was 2-4 °C below air temperature during the day and above air temperature in the early dark period (Figure 4. 1). The midday maximum PFD was 1600 μmol photons m^{-2} s^{-1} both in DCDW treatment and control experiments. The plants were moved to the Weiss chambers for adaptation to control conditions for 2 or 3 days before the 3 day DCDW treatment. After this chilling treatment, the environment was returned to control conditions for a period of recovery.

Light response curves of leaf photosynthesis in Phytotron grown plants were measured by O_2 exchange at CO_2 saturation in the leaf disc electrode as described in Section 2. 4. 1. Chlorophyll fluorescence at room temperature and at 77 K was measured as described in Section 2. 5. 1 and 2. 5. 2. The experiments on the effect of leaf orientation on fluorescence were done in natural field conditions and the controlled environment growth chambers in the GRRI, Guangzhou. Leaves were held horizontally with nylon thread frames similar to those used in laboratory

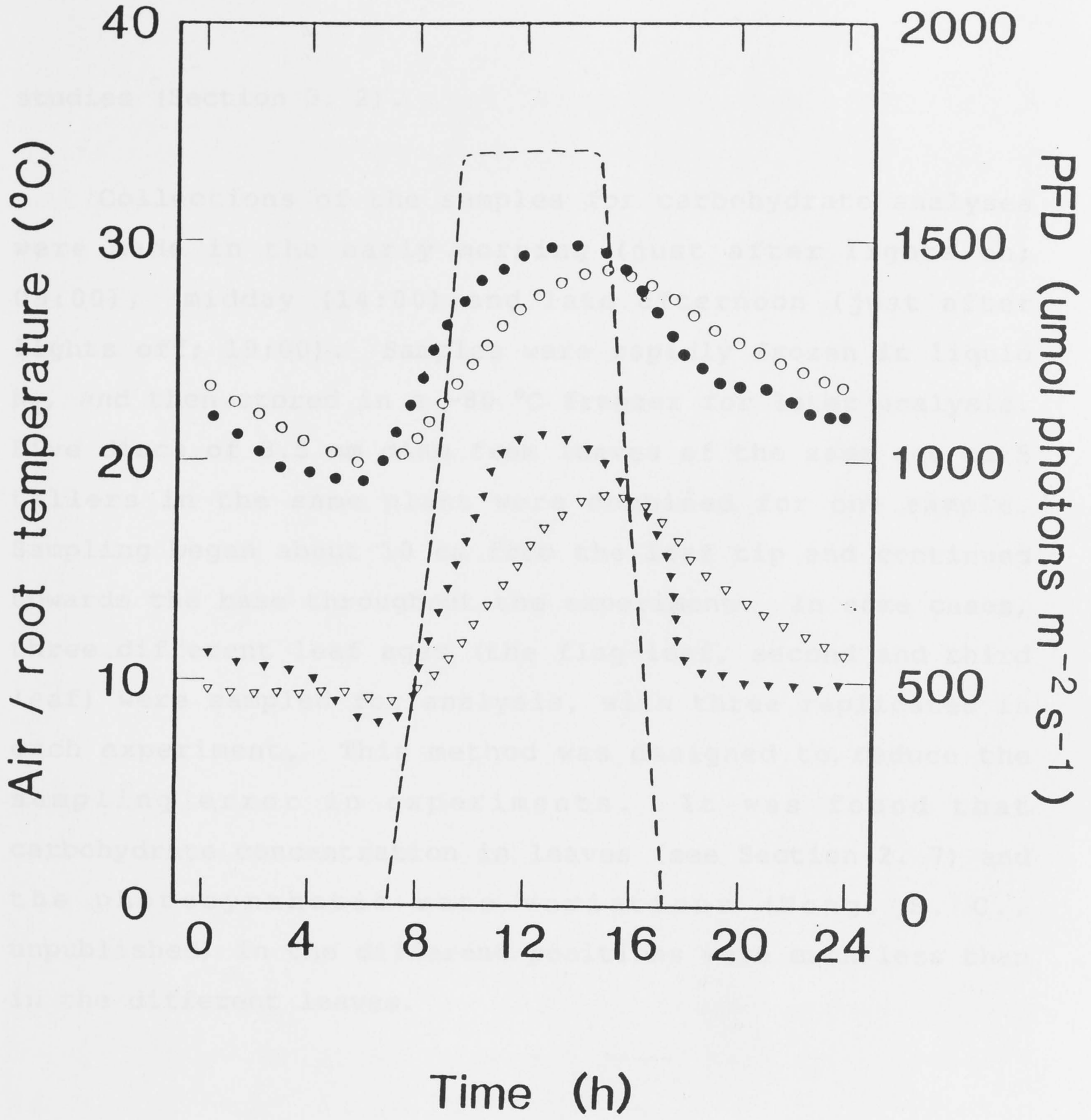

Figure 4. 1 Daily pattern of air and root temperature and PFD pattern of control and low temperature treatment in Weiss chamber. Symbols are: PFD (----), control air temperature (●), control root temperature (○), treatment air temperature (▼), treatment root temperature (∇).

studies (Section 3. 2).

Collections of the samples for carbohydrate analyses were made in the early morning (just after lights on; 09:00), midday (14:00) and late afternoon (just after lights off; 19:00). Samples were rapidly frozen in liquid N_2, and then stored in a -80 °C freezer for later analysis. Five discs of 3.5 mm diam from leaves of the same age on 5 tillers in the same plant were combined for one sample. Sampling began about 10 cm from the leaf tip and continued towards the base throughout the experiment. In some cases, three different leaf ages (the flag-leaf, second and third leaf) were sampled for analysis, with three replicates in each experiment. This method was designed to reduce the sampling error in experiments. It was found that carbohydrate concentration in leaves (see Section 2. 7) and the photosynthetic rate variations (Wong, S. C., unpublished) in the different positions were much less than in the different leaves.

4. 3. EXPERIMENTS AND RESULTS

4. 3. 1. Chlorophyll fluorescence and quantum yield of photosynthesis under simulated CDW conditions and in the field

The light response curve of photosynthetic O_2 evolution at CO_2 saturation and chlorophyll fluorescence at 77 K were measured twice a day (in early morning, 09:00 h, and late

afternoon at 19:00 h) in the leaves taken from plants in the Weiss chamber experiments described above. Quantum yield decreased throughout the chilling period in both cv. Hungarian-1 and Gui Chao-2 (Figure 4. 2). In cv. Hungarian-1 recovery was rapid and complete after chilling; in cv. Gui Chao-2 recovery was incomplete. There was little effect of chilling on the maximum rate of photosynthetic O_2 evolution in cv. Hungarian-1. However, in cv. Gui Chao-2, the maximum declined markedly early in the chilling period but recovered rapidly thereafter to show a significant diurnal change with higher rates in the morning than in the afternoon.

Small changes in the 77K fluorescence parameters were observed during these experiments (Figure 4. 3), and these were comparable to fluorescence changes measured at room temperature (data not shown). Surprisingly, cv. Gui Chao-2 showed very little response, but cv. Hungarian-1, which was less sensitive to low temperature photoinhibition in laboratory experiments, showed a large reversible increase in F_o and decrease in F_v/F_m on the first day following chilling which was reversed during the next night. However, after days 2 and 3 these changes persisted, and were not reversed until the chilling treatments ceased. In another experiment cv. Lemont was intermediate between these two responses (Figure 4. 4). The larger changes in the fluorescence parameters in cv. Hungarian-1 are almost certainly due to the fact that leaves of this cultivar are much more horizontally arranged than in cv. Gui Chao-2 and Lemont, and were therefore exposed to higher PFD in the chamber.

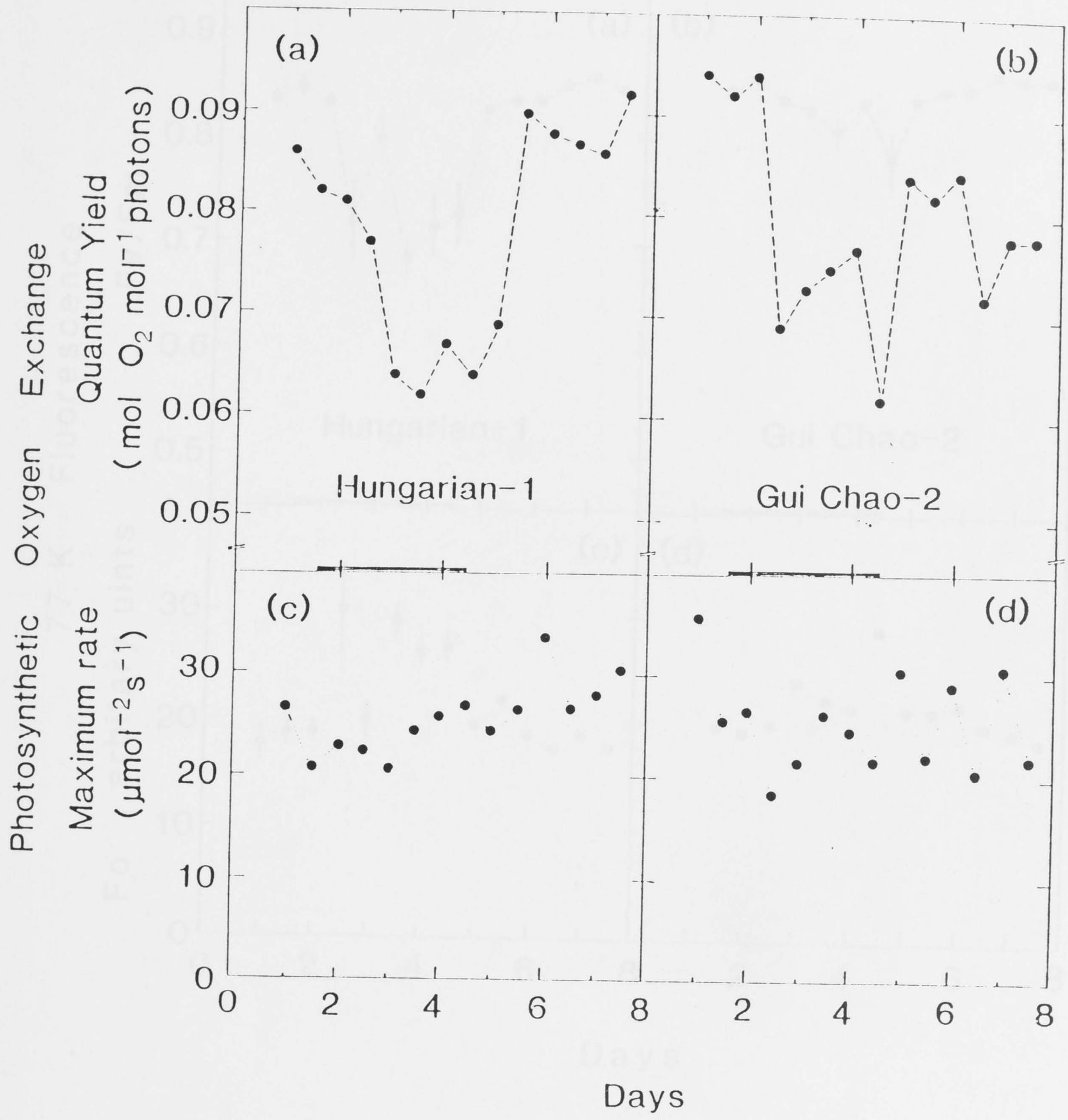

Figure 4. 2 Time course of changes in quantum yield (a and b) and maximum rate (c and d) of photosynthetic O_2 evolution at CO_2 saturation in leaves of cv. Hungarian-1 (a and c) and cv. Gui Chao-2 (b and d), measured twice (morning and afternoon) daily and throughout a simulated 3 day DCDW event.

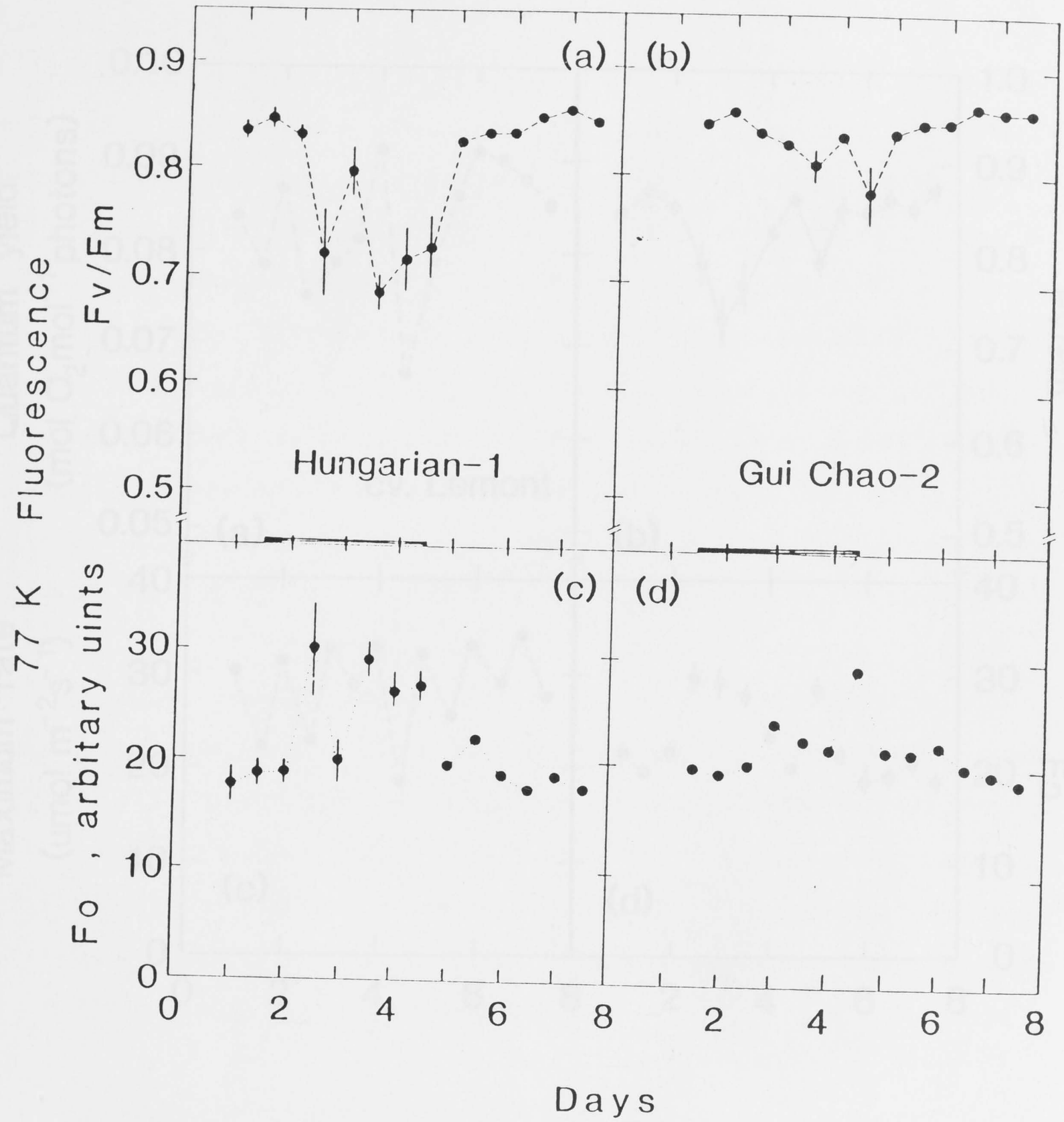

Figure 4. 3 Time course of changes in F_v/F_m (a and b) and F_o (c and d) of 77K fluorescence in leaves of cv. Hungarian-1 (a ad c) and cv Gui Chao-2 (b and d), measured twice (morning and afternoon) daily and throughout a simulated 3 day DCDW event.

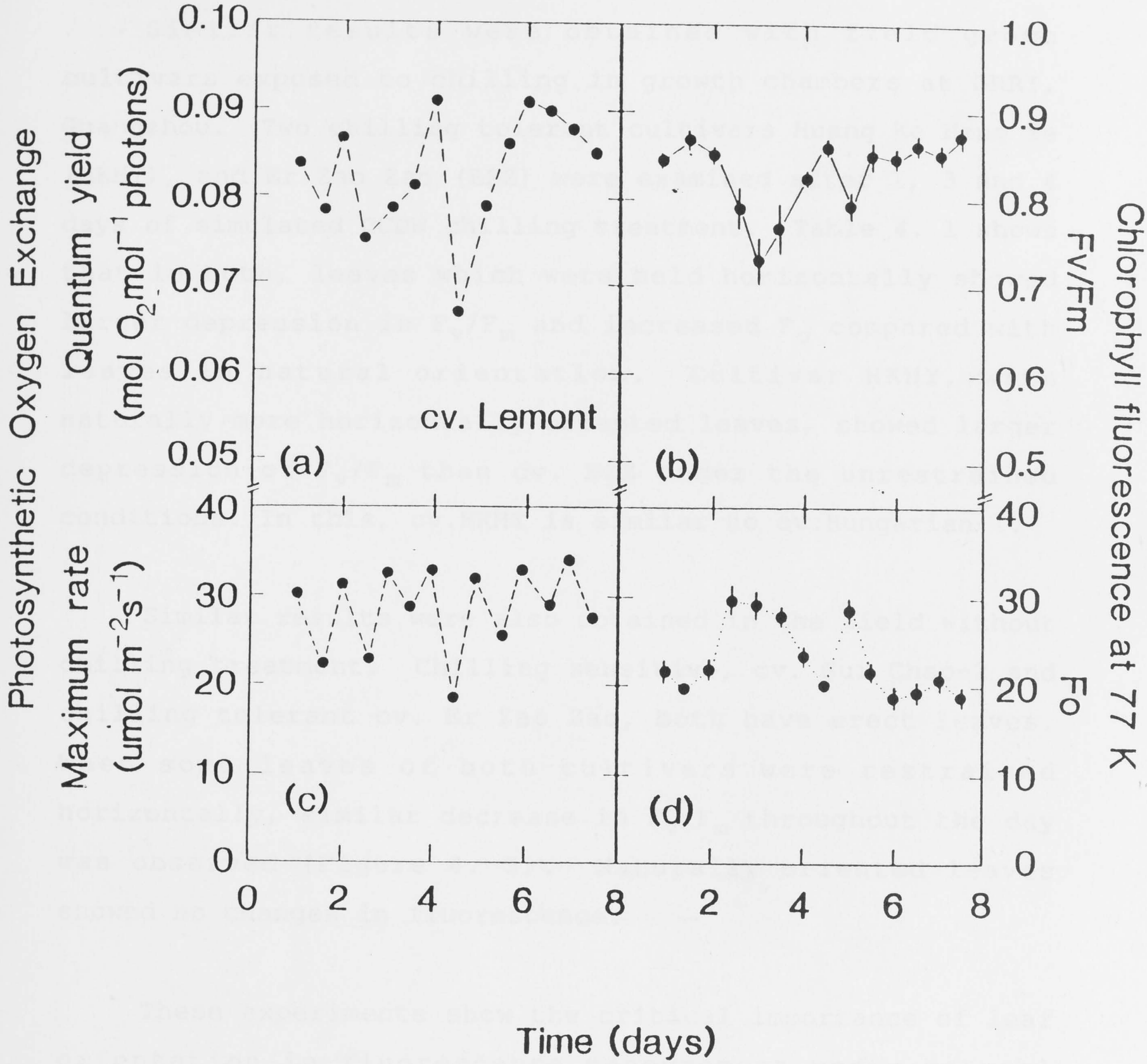

Figure 4. 4 Time course of changes in quantum yield (a), maximum rate of photosynthetic O_2 evolution at CO_2 (c), F_v/F_m (b) and F_o (d) of 77K fluorescence in leaves of cv. Lemont, measured twice (morning and afternoon) daily and throughout a simulated 3 day DCDW event.

Similar results were obtained with field grown cultivars exposed to chilling in growth chambers at GRRI, Guangzhou. Two chilling tolerant cultivars Huang Ke Heng Ye (HKHY), and Er Zao Zao (EZZ) were examined after 1, 3 and 4 days of simulated DCDW chilling treatment. Table 4. 1 shows that in both, leaves which were held horizontally showed larger depression in F_v/F_m and increased F_o compared with leaves in natural orientation. Cultivar HKHY, with naturally more horizontally oriented leaves, showed larger depression of F_v/F_m than cv. EZZ under the unrestrained conditions. In this, cv.HKHY is similar to cv.Hungarian-1.

Similar results were also obtained in the field without chilling treatment. Chilling sensitive, cv. Gui Chao-2 and chilling tolerant cv. Er Zao Zao, both have erect leaves. When some leaves of both cultivars were restrained horizontally, similar decrease in F_v/F_m throughout the day was observed (Figure 4. 5). Naturally oriented leaves showed no changes in fluorescence.

These experiments show the critical importance of leaf orientation in fluorescence measurement under natural conditions. As shown below, canopy photosynthesis was not correlated with fluorescence changes illustrated in Figure 4. 3. It seems unlikely that reduction in primary photosynthetic processes is responsible for reduced canopy photosynthesis under DCDW conditions.

Table 4. 1 Effects of low temperature on chlorophyll fluorescence parameters dependent on leaf orientation in different rice cultivars

Cultivars	Treatment	Horizontal 09:00	Horizontal 14:00	Natural orientation 09:00	Natural orientation 14:00
77K fluorescence F_v/F_m					
Huang Ke Heng Ye	control	0.887	-	0.886	-
		±0.017	-	±0.012	-
	chilling day 1	0.814	0.676	0.884	0.817
		±0.030	±0.013	±0.007	±0.007
	day 3	0.616	0.583	0.812	0.804
		±0.216	±0.138		±0.008
	day 4	0.517	0.478	0.836	0.809
		±0.189	±0.005	±0.037	±0.012
Er Zao Zao	control	0.886	-	0.893	-
		±0.006	-	±0.018	-
	chilling day 1	0.848	0.777	0.888	0.847
		±0.030	±0.063	±0.033	±0.025
	day 3	0.793	0.811	0.802	0.801
		±0.027	±0.067		±0.076
	day 4	0.836	0.809	0.758	0.792
		±0.037	±0.012	±0.025	
PAM (room temperature) fluorescence, F_v/F_m					
Huang Ke Heng Ye	control	0.636	-	0.818	-
		±0.018	-	±0.002	-
	chilling day 1	0.571	0.463	0.697	0.621
		±0.026	±0.010	±0.008	±0.026
	day 3	0.401	0.343	0.590	0.400
		±0.081	±0.065	±0.008	±0.016
	day 4	0.535	0.243	0.565	0.613
		±0.030	±0.076	±0.009	±0.036
Er Zao Zao	control	0.690	-	0.760	-
		±0.027	-	±0.020	-
	chilling day 1	0.635	0.554	0.719	0.666
		±0.031	±0.013	±0.005	±0.041
	day 3	0.624	0.441	0.628	0.487
		±0.010	±0.079	±0.025	±0.019
	day 4	0.660	0.663	0.056	0.613
		±0.044	±0.052	±0.009	±0.036

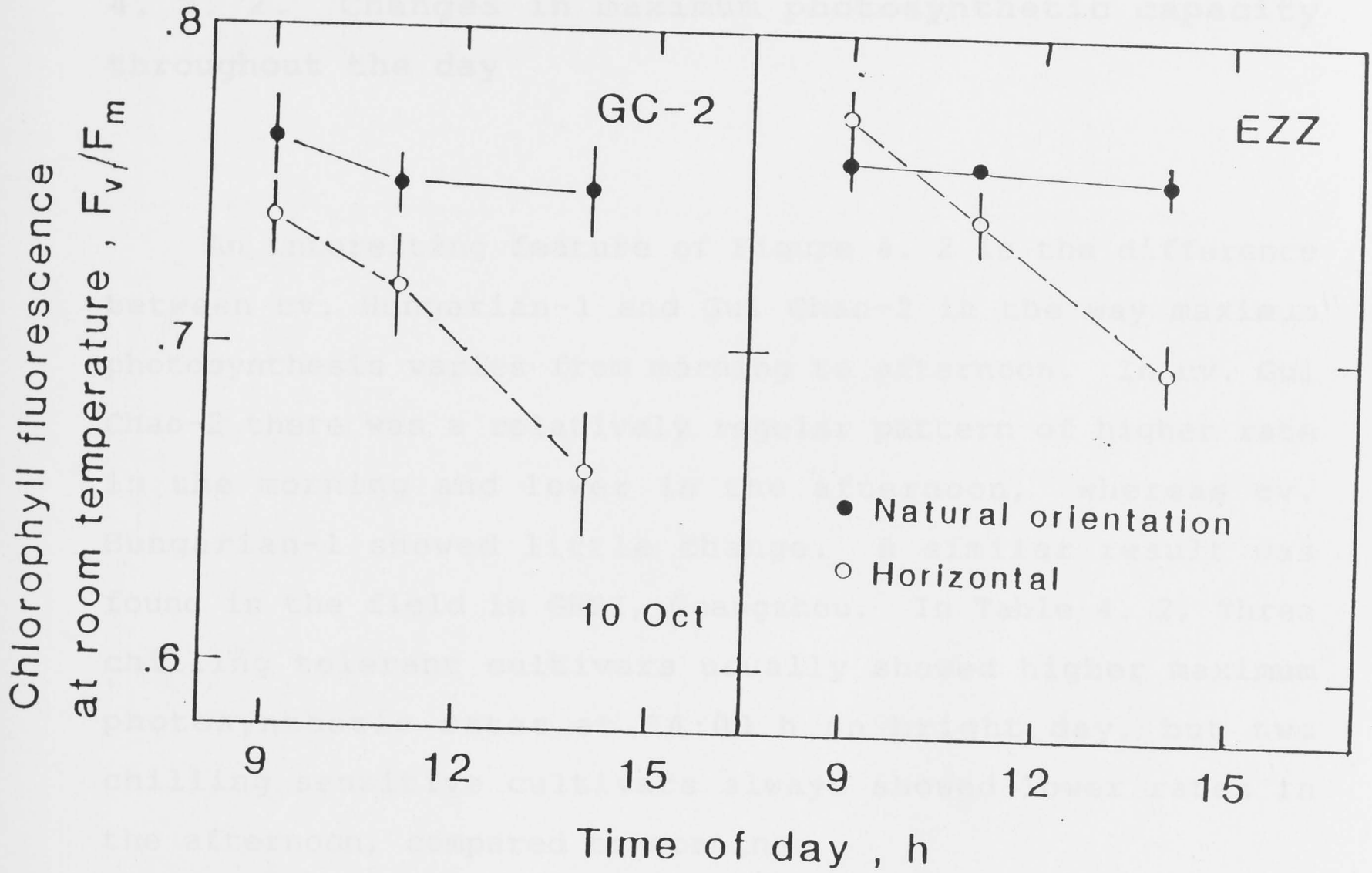

Figure 4. 5 Daily excursion in F_v/F_m of 77K fluorescence in leaves of field grown rice cultivars under natural (●) and horizontal (○) leaf orientations.

4. 3. 2. Changes in maximum photosynthetic capacity throughout the day

An interesting feature of Figure 4. 2 is the difference between cv. Hungarian-1 and Gui Chao-2 in the way maximum photosynthesis varies from morning to afternoon. In cv. Gui Chao-2 there was a relatively regular pattern of higher rate in the morning and lower in the afternoon, whereas cv. Hungarian-1 showed little change. A similar result was found in the field in GRRI, Guangzhou. In Table 4. 2, Three chilling tolerant cultivars usually showed higher maximum photosynthetic rates at 14:00 h on bright day, but two chilling sensitive cultivars always showed lower rates in the afternoon, compared to morning.

Examination of earlier experiments using phytotron grown plants, showed that the maximum rates of photosynthesis were 20-30 % lower when measured in the late afternoon, compared with measurements in the early morning (Figure 4. 6). One clear difference in the composition of rice leaves between morning and evening is the soluble sugars content (Figure 2. 7 and Table 4. 3). These differences were observed in all experiments with rice plants grown in controlled environments (see below). They suggest that photosynthesis in rice plants may be very sensitive to soluble sugar accumulation, even under normal growing conditions.

Table 4. 2 Maximum photosynthesis and quantum yield in rice cultivars growing under optimal field conditions in Guangzhou

Cultivars	Date	Maximum photosynthesis (μmol O_2 m^{-2} s^{-1})	
		06:00	14:00
Hungarian-1	6/10	31.6	44.4
Er Zao Zao	8/10	51.1	58.0
	13/10	36.4	37.8
	18/10	45.1	40.2
Huang Ke Heng Ye	12/10	54.3	53.6
	17/10	55.0	43.2
Wan Zhu-3	8/10	52.3	43.2
	13/10	47.8	33.1
	18/10	52.3	44.6
Gui Chao-2	6/10	43.2	28.8
	12/10	46.0	32.7
	17/10	50.9	38.9

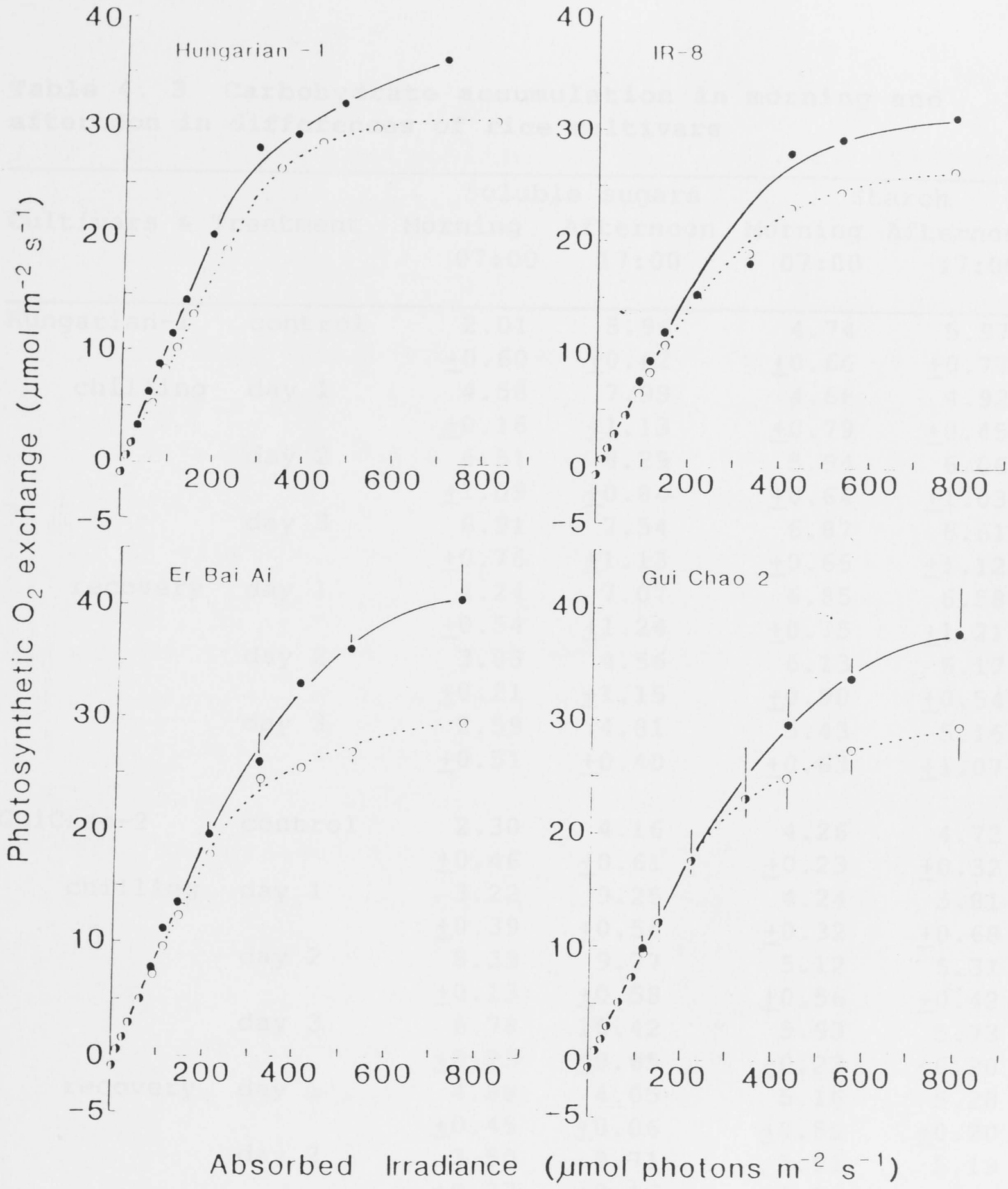

Figure 4. 6 The light response curves of photosynthetic O_2 evolution in four rice cultivars measured on leaves collected early morning (●) or late afternoon (○). Plants were grown in the CSIRO Phytotron. The mean incident solar energy fluxes (MJ m^{-2} d^{-1}) for the 10 days prior to the experiments reported, and that of day of measurement (in parenthesis) were as follows: Hungarian-1, 23.9±3.2 (24.5); IR-8, 19.2±2.7 (21.0); Er Bai Ai, 21.1±5.8 (17.8); and Gui Chao-2, 19.4±3.8 (19.1).

Table 4. 3 Carbohydrate accumulation in morning and afternoon in differences of rice cultivars

Cultivars & Treatment		Soluble sugars		Starch	
		Morning 07:00	Afternoon 17:00	Morning 07:00	Afternoon 17:00
Hungarian-1	control	2.01	5.96	4.74	5.97
		±0.60	±0.62	±0.66	±0.77
chilling	day 1	4.58	7.99	4.66	4.92
		±0.16	±1.13	±0.79	±0.45
	day 2	6.51	8.29	5.94	6.06
		±1.09	±0.84	±0.64	±1.03
	day 3	6.91	7.54	6.87	6.61
		±0.76	±1.13	±0.65	±1.12
recovery	day 1	4.24	7.07	6.85	6.58
		±0.54	±1.24	±0.75	±1.21
	day 2	3.03	4.56	6.13	6.17
		±0.21	±1.15	±0.90	±0.54
	day 3	2.59	4.81	5.43	5.16
		±0.51	±0.40	±0.63	±1.07
GuiChao-2	control	2.30	4.16	4.26	4.72
		±0.46	±0.61	±0.23	±0.32
chilling	day 1	3.22	9.25	4.24	5.81
		±0.39	±0.54	±0.32	±0.68
	day 2	8.33	9.77	5.12	5.31
		±0.13	±0.58	±0.56	±0.42
	day 3	8.78	10.42	5.93	5.73
		±0.23	±0.65	±0.23	±0.20
recovery	day 1	4.88	4.05	5.16	5.28
		±0.45	±0.06	±0.51	±0.20
	day 2	3.50	3.71	5.01	5.19
		±0.27	±0.16	±0.19	±0.41
	day 3	2.62	2.75	4.71	4.55
		±0.09	±0.27	±0.08	±0.11
IR-8	control	4.02	5.87	4.26	4.75
		±0.61	±0.56	±0.19	±0.61
chilling	day 1	5.42	14.68	4.71	5.31
		±0.14	±0.22	±0.47	±0.83
	day 2	13.20	15.98	4.98	4.27
		±0.25	±1.07	±0.27	±0.34
	day 3	14.19	15.38	4.74	4.90
		±0.88	±0.76	±0.31	±0.41
recovery	day 1	10.62	-	4.53	-

4. 3. 3. Canopy photosynthesis and leaf carbohydrate composition under simulated DCDW conditions

The control and recovery conditions selected for the controlled environment studies were based on those measured on 16 October, 1986, and those selected as representative of DCDW conditions, based on those measured on 30 October, 1986 (Figure 2. 1). The results of an experiment with cv. Er Bai Ai in which root temperatures were allowed to follow air temperature is shown in (Figure 4. 7). The first day of the chilling treatment caused very little decrease in the canopy photosynthesis, but on day 2 and day 3, net photosynthesis at mid-day declined by 25% and 57% respectively. In this and all other experiments, dark respiration declined significantly during the chilling, but increased markedly again during the recovery, exceeding that of the initial control rate by up to 50%, especially during the early part of the dark period. After 3 days of the the recovery under control conditions, canopy photosynthesis returned to about 63% of its initial value.

Cultivars differed in their response to these treatments. Canopy photosynthesis in cv. Hungarian-1 and Lemont was less sensitive to the above treatments than cv. Gui Chao-2, Er Bai Ai and IR-8 (Table 4. 4).

The concentration of carbohydrates in rice leaves was measured during the canopy photosynthesis experiments in the Weiss chambers. Sampling was done three times daily at 09:00, 14:00, and 19:00. Simulated DCDW conditions are as above.

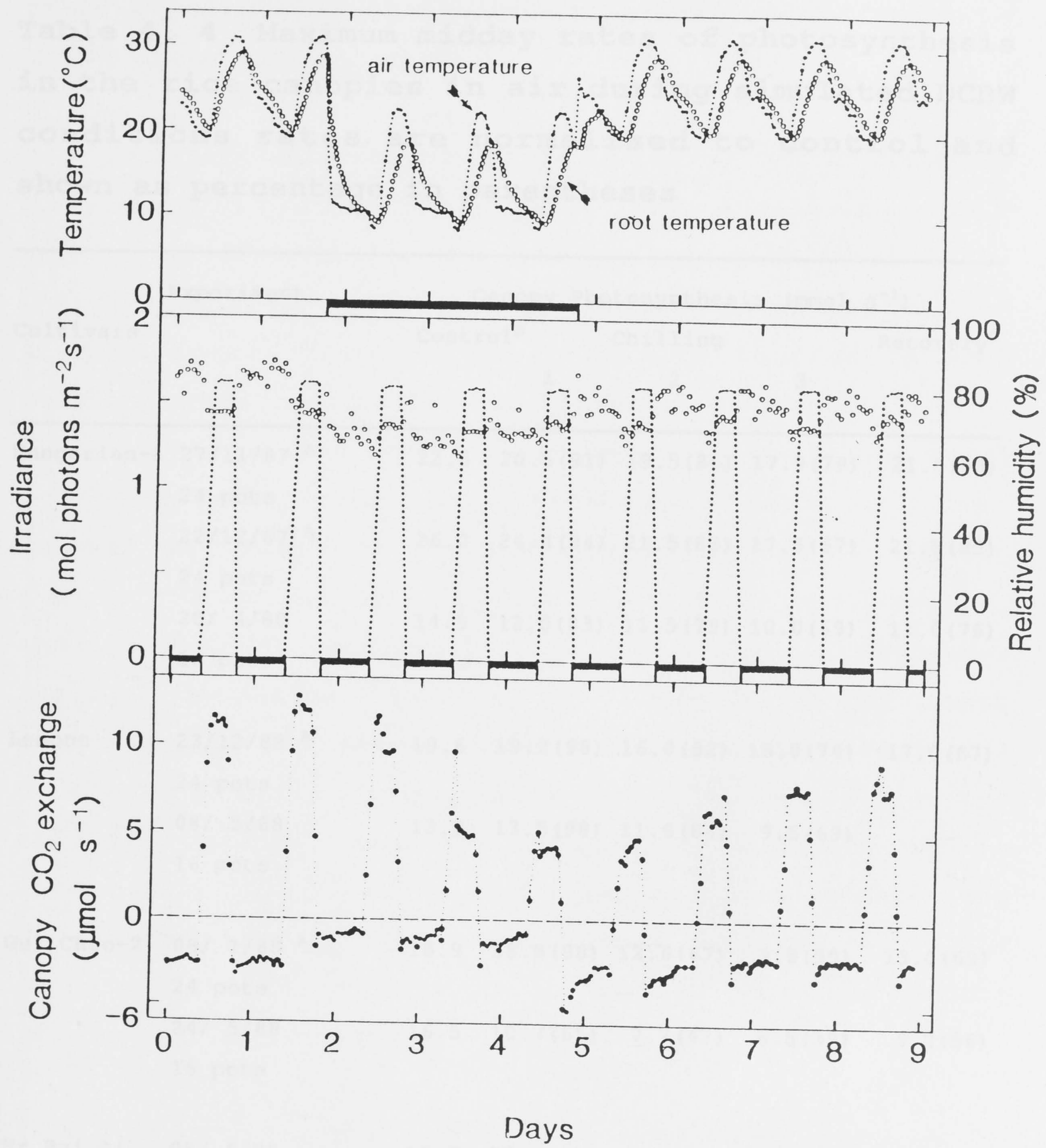

Figure 4. 7 Environmental variables and canopy photosynthesis measured in closed control environment chambers throughout a simulated 3 day DCDW event with cv. Er Bai Ai.

Table 4. 4 Maximum midday rates of photosynthesis in the rice canopies in air during simulated DCDW conditions rates are normalised to control and shown as percentage in parentheses

Cultivars	Experiment	Canopy Photosynthesis (mmol s^{-1})				
		Control[B]	Chilling			Recovery[C]
			1	2	3	
Hungarian-1	27/11/87 [A] 24 pots	22.6	20.5(91)	19.5(86)	17.9(79)	21.0(93)
	22/12/87 [A] 24 pots	26.0	24.4(94)	21.5(83)	17.5(67)	21.5(83)
	20/ 4/88 16 pots	14.5	12.0(83)	11.5(79)	10.0(69)	11.0(76)
Lemont	23/12/88 [A] 24 pots	19.6	19.2(98)	16.0(82)	15.0(76)	17.0(87)
	08/ 5/88 16 pots	13.8	13.5(98)	11.6(84)	9.5(69)	–
Gui Chao-2	08/ 1/88 [A] 24 pots	18.9	18.5(98)	12.6(67)	9.5(50)	13.0(69)
	24/ 5/88 16 pots	16.5	10.7(65)	7.7(47)	6.5(39)	9.2(56)
Er Bai Ai	08/ 5/88 16 pots	12.7	12.1(95)	9.5(75)	5.4(43)	8.0(63)
IR-8	04/ 6/88 16 pots	15.3	12.2(80)	8.1(53)	5.0(33)	–

[A] Roots kept at 20 °C.

[B] Average of 2 days before chilling.

[C] Average of 2 days, 2 days after chilling ceased.

These experiments were quite comparable with respect to canopy photosynthesis rates.

In a less chilling-sensitive cultivar, cv. Hungarian-1, the changes in concentration of soluble sugars and starch were quite comparable to the chilling sensitive rice cv. IR-8 and Gui Chao-2. On the first chilling night the loss of soluble sugars from the leaf was much reduced in both chilling-sensitive and chilling insensitive rice cultivars. All cultivars accumulated soluble sugars to about same total concentration by the end of the first chilling day. However, cv. Hungarian-1 showed a rapid increase of soluble sugars to a maximum level by the end of first chilling day, and a subsequent decrease of this concentration throughout the chilling period (Figure 4. 8). Concentration of starch accumulated in the leaves showed a slow increase, beginning by the first chilling day, which was maintained during the chilling period. When the environment was returned to normal condition leaves showed a return to large day/night changes in soluble sugars and starch declined. In this experiment, photosynthesis did not recover, possibly because of nutrient limitations.

In contrast, the concentration of soluble sugars in leaves of chilling-sensitive rice cv. IR-8 increased by the end of the first chilling day, and remained high throughout the 3 day chilling period. The starch content in leaves was not different during chilling and recovery periods, compared with the initial control (Figure 4. 9). The trend of changes in concentration of soluble sugars and starch accumulated were similar in leaves of different ages.

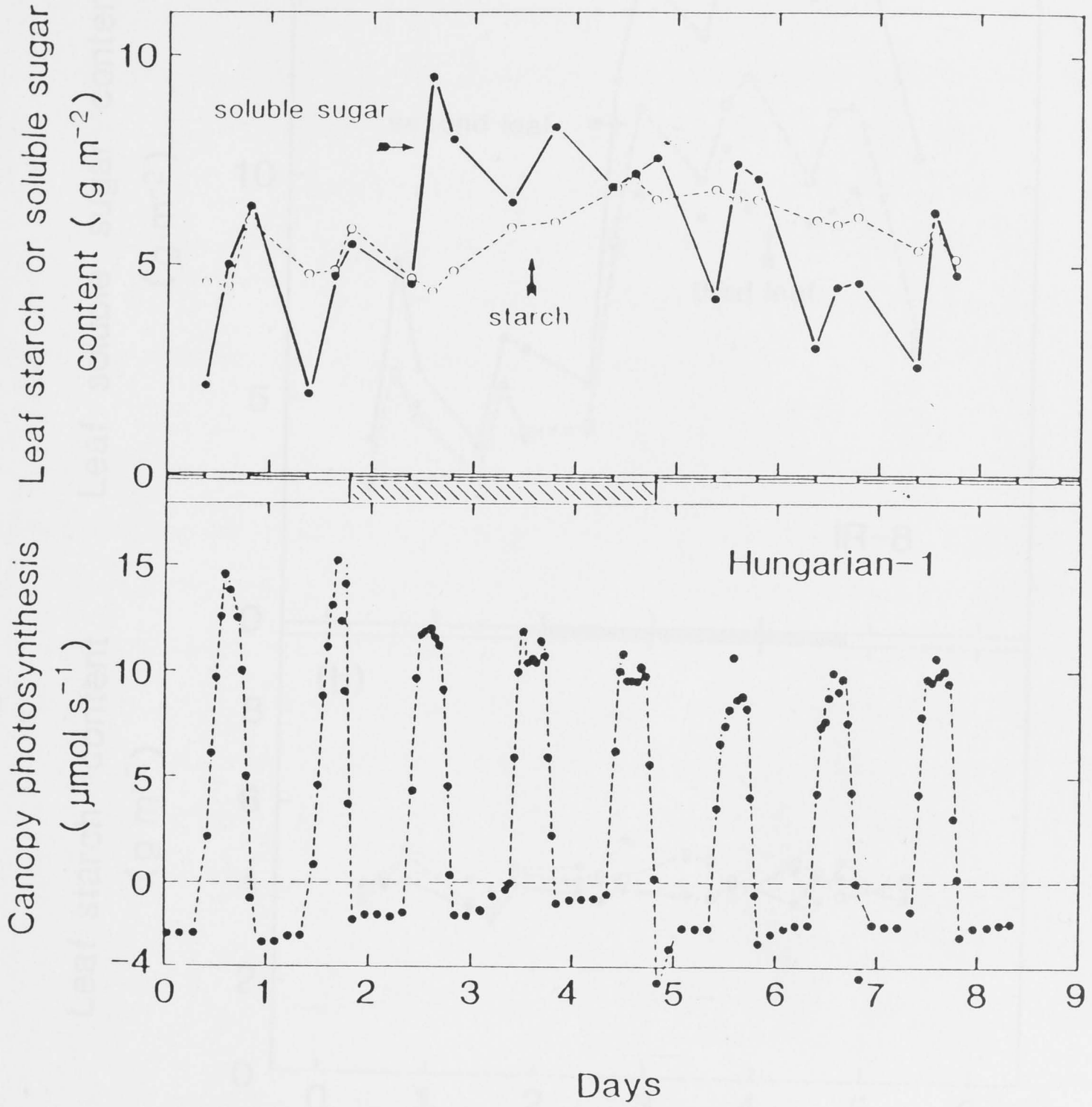

Figure 4. 8 Time course of canopy photosynthesis and the concentrations of soluble sugars (●) and starch (○) accumulation in leaves of cv. Hungarian-1 throughout simulated 3 day DCDW event.

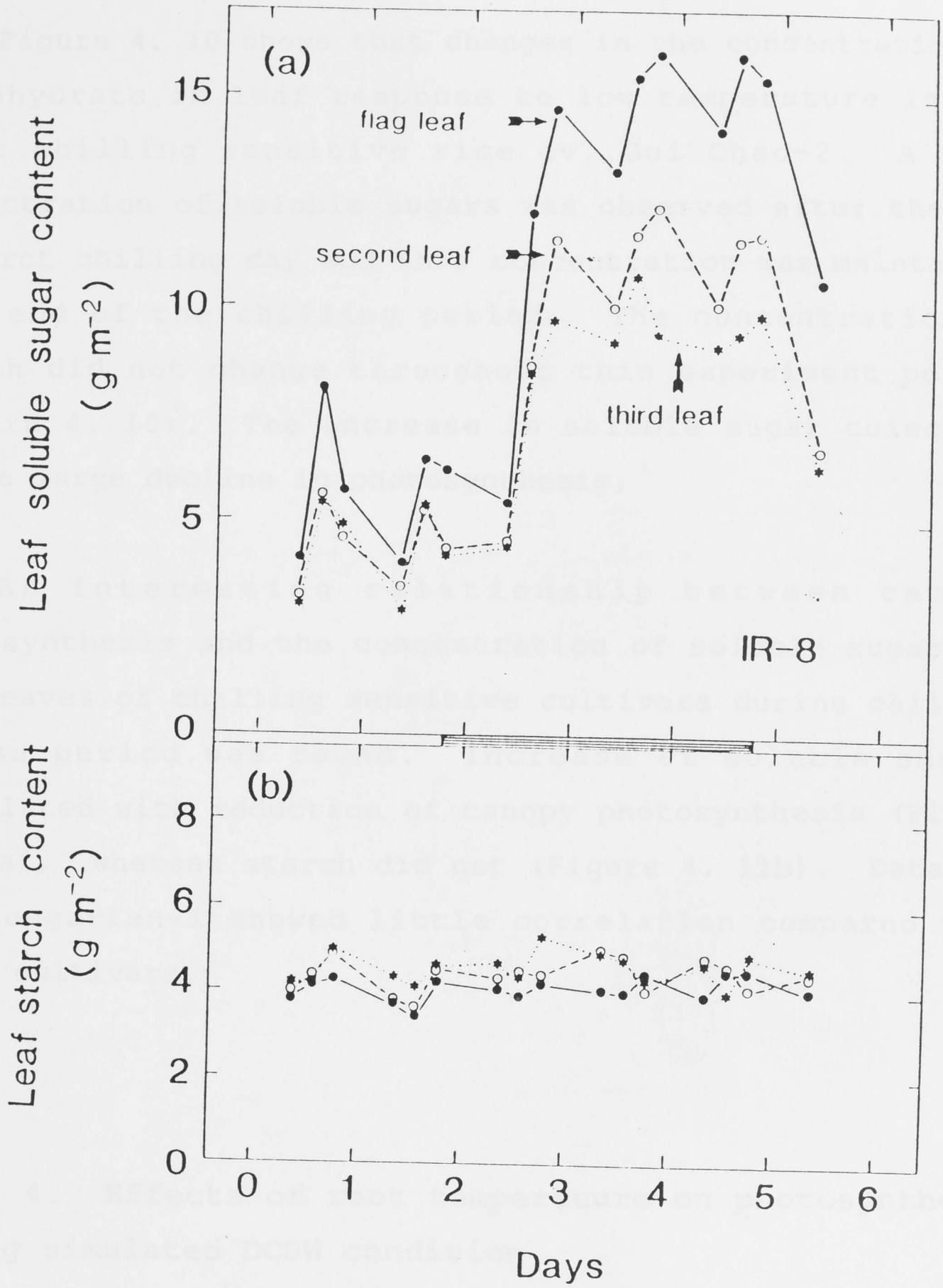

Figure 4. 9 Time course of changes in soluble sugars and starch concentrations in leaves of cv. IR-8 throughout simulated 3 day DCDW event.

Figure 4. 10 shows that changes in the concentration of carbohydrate in leaf response to low temperature in the other chilling sensitive rice cv. Gui Chao-2. A high concentration of soluble sugars was observed after the end of first chilling day and this concentration was maintained till end of the chilling period. The concentration of starch did not change throughout this experiment period (Figure 4. 10). The increase in soluble sugar coincided with a large decline in photosynthesis.

An interesting relationship between canopy photosynthesis and the concentration of soluble sugars in the leaves of chilling sensitive cultivars during chilling stress period was found. Increase of soluble sugars correlated with reduction of canopy photosynthesis (Figure 4. 11a), whereas starch did not (Figure 4. 11b). Data for cv. Hungarian-1 showed little correlation compared with other cultivars.

4. 3. 4. Effects of root temperature on photosynthesis during simulated DCDW condition

The extent of the depression in photosynthesis throughout the chilling treatment was reduced when the roots of the plants were maintained at 20 °C (air temperature was unaffected by this treatment). This treatment had a relatively large effect on the extent of reduction in canopy photosynthesis during chilling of cv. Hungarian-1 (Figure 4. 12a), but less additional effect in cv. Gui Chao-2

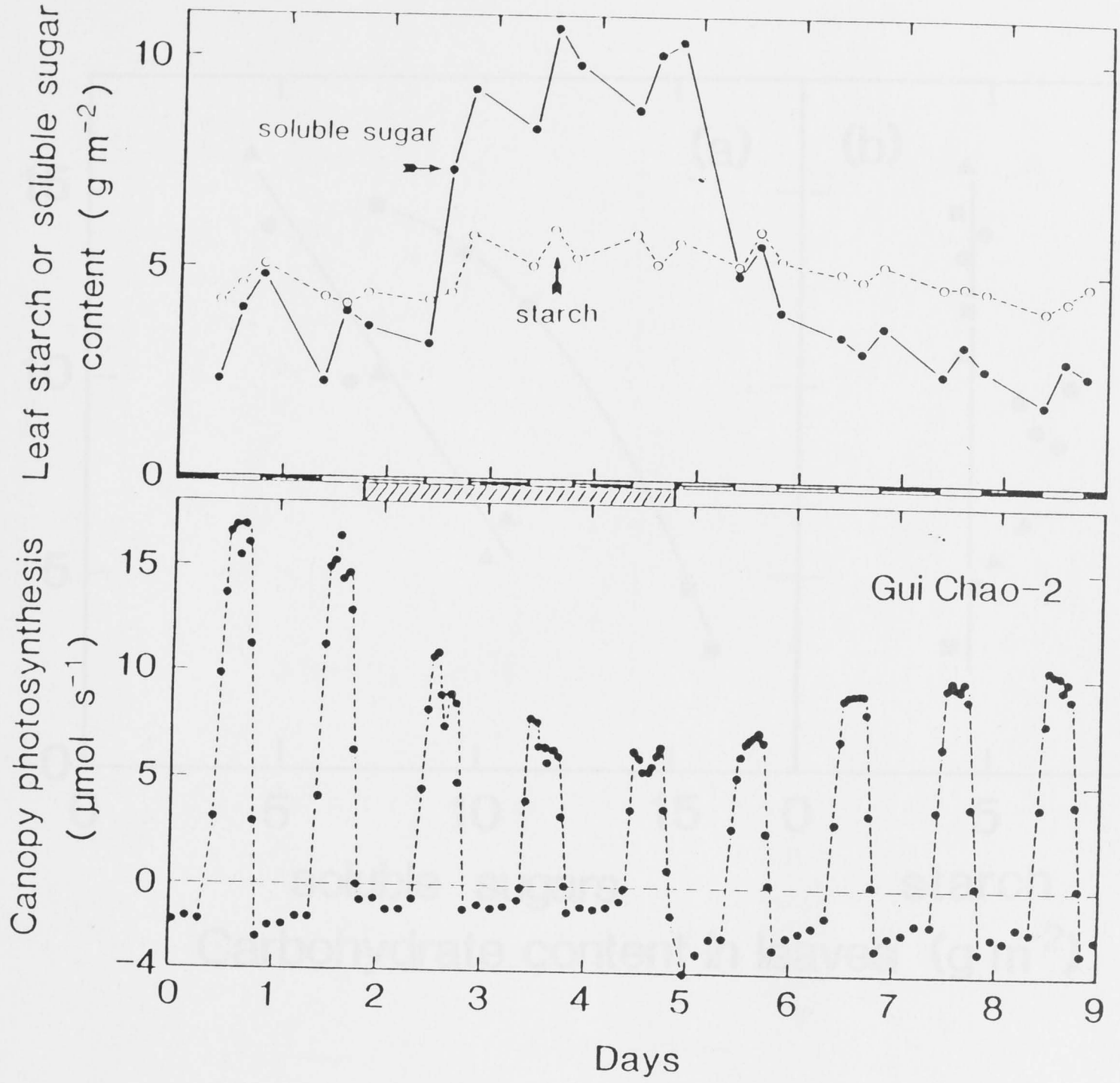

Figure 4. 10 Time course of canopy photosynthesis and the concentrations of soluble sugars (●) and starch (○) accumulation in leaves of cv. Gui Chao-2 throughout simulated 3 day DCDW event.

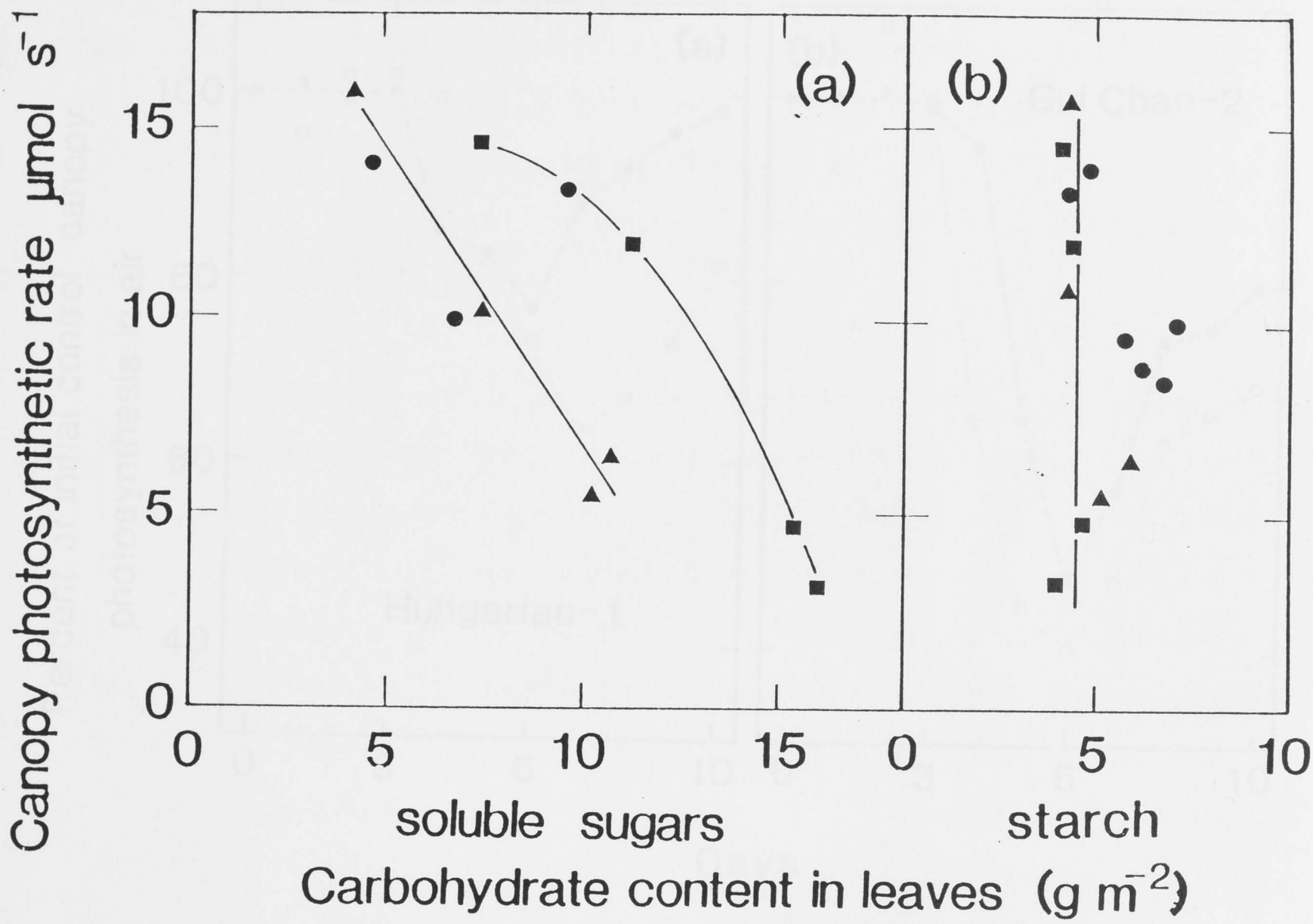

Figure 4. 11 Soluble sugars content (a) and starch content (b) in the leaves of cv. Hungarian-1 (●), Gui Chao-2 (▲), and IR-8 (■) plotted against canopy photosynthetic rate during low temperature treatment.

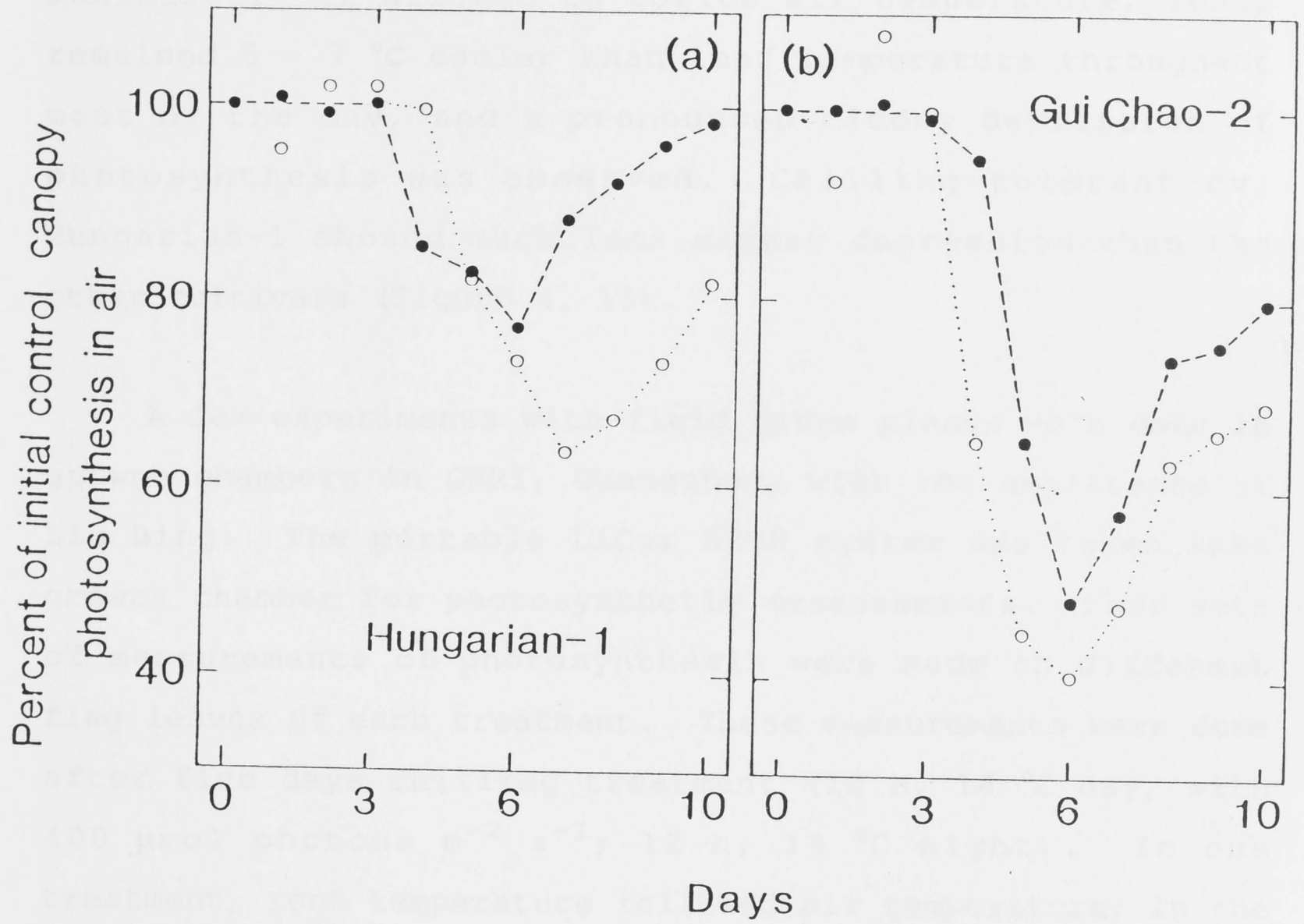

Figure 4. 12 Effect of root temperature on extent of chilling-induced inhibition of canopy photosynthesis. Normalised average midday canopy photosynthesis (12:00-14:00 h) for (a) cv. Hungarian-1 and (b) cv Gui Chao-2 with roots kept at 20 °C (●) or allowed to follow air temperature (○) throughout a simulated 3 day DCDW event.

(Figure 4. 12b). With warm roots the differences in chilling responses between these two cultivars were maximised. If allowed to follow air temperature, roots remained 5 - 7 °C cooler than leaf temperature throughout most of the day, and a pronounced midday depression of photosynthesis was observed. Chilling-tolerant cv. Hungarian-1 showed much less midday depression than the other cultivars (Figure 4. 13).

A few experiments with field grown plants were done in growth chambers in GRRI, Guangzhou, with the assistance of Liu Bing. The portable LiCor 6200 system was taken into growth chamber for photosynthetic measurements. Four sets of measurements of photosynthesis were made on different flag leaves of each treatment. These measurements were done after five days chilling treatment (12 h, 14 °C day, with 400 μmol photons m^{-2} s^{-1}; 12 h, 14 °C night). In one treatment, root temperature followed air temperature; in the other roots were kept at about 20 °C by flowing tap water. All cultivars showed reduction of photosynthesis, associated with stomatal closure, in plants with cold roots (Table 4. 5).

In addition to reducing the extent of chilling-induced inhibition of photosynthesis, warming of the roots abolished a mid-day depression of photosynthesis which was observed during chilling treatments (Figure 4. 13). Similar results were obtained with all cultivars. All of these experiments show that a change in weather conditions equivalent to the DCDW can drastically impair canopy photosynthesis in rice. Cultivars differ in the extent to which photosynthesis is impaired, and the response is exaggerated if root

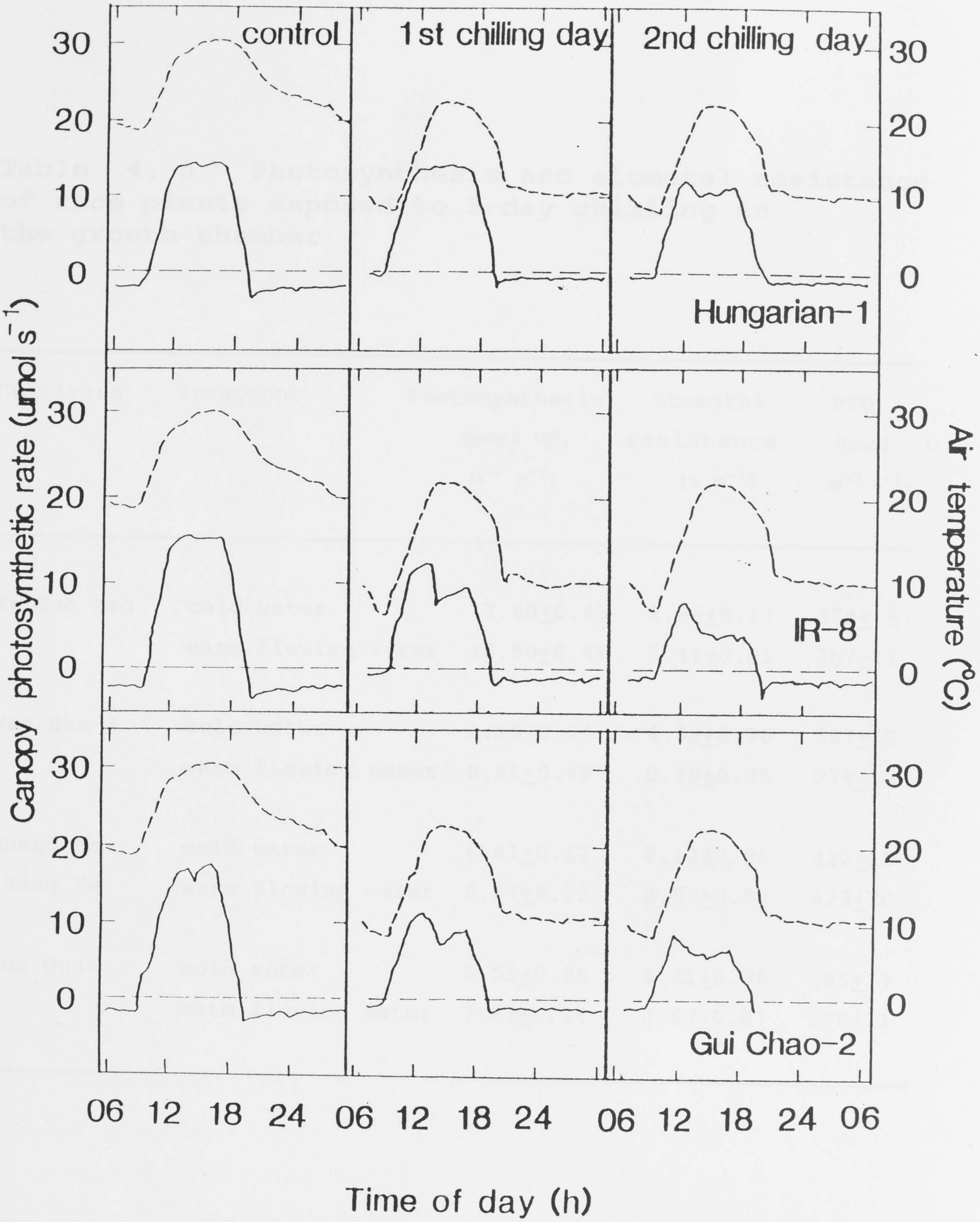

Figure 4. 13 Time course of air temperature and canopy photosynthetic rate of control and low temperature treatment of three rice cultivars; air temperature (----), and canopy photosynthesis (——).

Table 4. 5 Photosynthesis and stomatal resistance of rice plants exposed to 5-day chilling in the growth chamber

Cultivars	Treatment	Photosynthesis (μmol CO_2 m^{-2} s^{-1})	Stomatal resistance (s m^{-1})	PFD (μmol m^{-2} s^{-1})
Er Zao Zao	cold water	7.60±0.43	2.86±0.17	364± 5
	warm flowing water	10.90±0.48	0.49±0.01	357±11
Wan Zhu-3	cold water	4.15±0.34	4.88±0.76	387±15
	warm flowing water	8.21±0.49	0.49±0.05	374±12
Huang Ke-Heng Ye	cold water	6.81±0.22	2.12±0.25	412±18
	warm flowing water	8.57±0.22	0.62±0.07	423±20
Hua Quan	cold water	5.55±0.26	3.21±0.35	385± 7
	warm flowing water	7.67±0.44	0.67±0.07	378±11

temperature is allowed to follow air temperature.

4. 4. DISCUSSION

These experiments show that photosynthesis in the canopies of the rice plants, treated and measured during the heading/flowering to grain filling stages (50-80 % of the tillers anthesis) was sensitive to chilling. Cultivars varied in their sensitivity to chilling. A Japonica-like rice, cv. Hungarian-1 from central Europe, and a modern Indica rice, cv. Lemont from Texas, were less sensitive to a chilling event which simulates DCDW of South-East China than were Chinese cultivars, Indica-derived rice cv. Gui Chao-2 and Er Bai Ai. Of the cultivars tested, the modern high yielding, Indica rice, cv. IR-8 was the most sensitive to a 3-day chilling treatment chosen to simulate a short, but severe chilling event comparable to conditions measured in the field at Guangzhou in October 1986.

Photosynthetic quantum yield and 77K chlorophyll fluorescence parameters were also measured during the simulated DCDW period. Paradoxically, increases in the initial fluorescence (F_o) and decreases in the ratio of the variable to maximum fluorescence (F_v/F_m) in chilling-tolerant rice, cv. Hungarian-1 under simulated DCDW conditions, which reflect differences in leaf orientation and light interception in the different cultivars, are not associated with significant changes in photosynthetic CO_2 assimilation. Thus it seems that changes in the efficiency

of photochemical reaction centre of PS II are not responsible for reduced photosynthesis of the rice canopy in response to chilling stress during the simulated DCDW event.

As shown in Figures 4. 2 and 4. 3, cv. Hungarian-1, the cultivar which showed least impairment of canopy photosynthesis showed greater reduction in quantum yield and larger changes in low temperature fluorescence parameters during chilling treatments periods. These changes were readily reversible and may be related to the more horizontal leaf habit of this cultivar compared with the more vertical orientation of leaves in the other cultivars. As shown in Figure 4. 5, leaves of both chilling-insensitive rice cv. Er Zao Zao and chilling-sensitive rice cv. Gui Chao-2 displayed similar daily changes in F_v/F_m when kept horizontal under field conditions. Table 4. 1 showed that, in two chilling-insensitive rice, cv. Huang Ke Heng Ye (a tall cultivar) and Er Zao Zao (a short compared with HKHY) exposed to simulated DCDW stress conditions, in which leaves of both cultivars were either held horizontal or natural orientation. The natural orientation showed much less photoinhibition.

Normally, the PFD incident on rice leaves in the natural orientation is only about 10-20 % compared with horizontal leaves, and this is unlikely to lead to photoinhibition. Moreover, most leaves in a rice canopy are shaded, so that it seems unlikely that photoinhibition will be observed in rice canopies in the field. Thus, the use of fluorescence to indicate chilling-tolerance in rice cultivars under field conditions is unlikely to be helpful except in tall cultivars with some horizontal leaves.

As shown in Table 4. 3, soluble sugars in leaves of rice increased in the afternoon compared with the morning, in all experiments with rice plants grown in controlled environments. This was seen in the normal PFD glasshouse grown rice plants in Canberra (as will be described in Chapter 5) as well in the natural field conditions in Guangzhou (data not shown). These results suggest that photosynthesis in rice plants may be very sensitive to soluble sugar accumulation, even under normal growing conditions.

In experiments with simulated DCDW in the Weiss chamber, three cultivars tested show a 3-fold diurnal increase in soluble sugars concentration in leaves, and a corresponding nocturnal decrease under control conditions. In these cultivars the first chilling night almost totally impaired leaf sucrose utilisation. The next day, soluble sugars increased to about five times the normal morning concentration. The accumulation of soluble sugars improves leaf cell water status and could help offset water stress if it develops when roots are cold. However, sucrose accumulation is clearly associated with a large decrease in photosynthesis in the cultivars, cv. Gui Chao-2 and IR-8. In cv. Hungarian-1, the initial increase is comparable, but leaf soluble sugars then tend to decline, and starch tends to increase throughout the chilling treatment, with little reduction of photosynthesis.

The cause of soluble sugars accumulation coincident with a decreased rate of photosynthesis is not known. It may reflect the temperature sensitivity of translocation and/or respiration. Soluble sugars remained in leaves

during the first chilling night, suggesting impaired translocation. Reduced respiration at low temperature might also contribute to maintenance of high leaf soluble sugar levels. However, we estimate that reduced respiration might conserve about 4 g sucrose in these canopies, only a small proportion of the total retained in leaves at low temperature (about 25 g). Nevertheless respiration is very responsive to these treatments, as indicated by the massive decline in soluble sugars and increased rate of respiration in the first night after relief of chilling. Whatever the cause, cooling the roots of some plants leads to substantial decreases in photosynthesis which are presumed to be associated with soluble sugars accumulation in the source leaves (Bagnall, *et al.*, 1988).

The causes and mechanisms of accumulation of a high concentration of soluble sugars in the leaves by the first chilling day are not known. It has been conjectured that short-term accumulation of soluble sugars in leaves is an adaptation to low temperature stress conditions. However, in the long-term, the situation is different. It has long held that soluble sugars accumulation in leaves has a feedback, inhibitory effect on the rate of photosynthesis. There is much evidence that photosynthesis can be impaired if the rate of sucrose synthesis in the cytoplasm is accelerated under conditions of rapid export (Stitt *et al.*, 1984; Huber, 1986). It is believed that high concentrations of phosphorylated intermediates needed to sustain sucrose synthesis in the cytoplasm lead to an imbalance in inorganic phosphate supply to the chloroplast and a decrease in photosynthetic rate. The mechanism of this model is based on studies of the inhibitory effects of artificial

cytoplasmic phosphate sequestering agents such as D-mannose on photosynthesis (Herold, 1980).

However, this model is not directly applicable to the situation described here, or in girdling experiments when sucrose accumulates to high concentrations in leaves because its translocation is impaired. Foyer (1987) proposed an "overflow" mechanism, based on hydrolysis of accumulated sucrose to glucose and fructose. The latter compounds then sequester phosphate in the cytosol and impair photosynthesis. Interestingly, Azcón-Bieto (1983) found that in wheat, all three soluble sugars (sucrose, glucose and fructose) increased on girdling. Phosphorylated sugars have not been measured in such experiments, but they do increase markedly in short term experiments (Leegood, unpublished). Of even greater interest perhaps, are the observation of Akita and Miyasaka (1969) and Tanaka and Yoshitomi (1973) that, following chilling, photosynthesis in rice is inhibited by low O_2. This response, counter-intuitive with respect to photorespiratory processes, is somewhat diagnostic of phosphate limited photosynthesis (Sharkey, 1985; Sage and Sharkey, 1987).

As shown in Figure 4. 12, low root temperature exaggerated effects of chilling stress in rice, and the extent of depression in maximum rate of photosynthesis during chilling treatment was reduced if the roots of treated plants were maintained at 20 °C.

Results of controlled environment chamber experiments with warm and cold roots supported these experiments (see Table 4. 5). These results suggest that low root

temperature also led to increased stomatal resistance. Thus it is necessary to make careful observations of leaf transpiration to determine if stomatal closure is a cause of reduced photosynthesis under CDW conditions, before concluding that metabolic regulation following sucrose accumulation is the main cause of low temperature inhibition of photosynthesis in rice.

Other Australian field observations suggest water stress may occur in rice leaves in bright light after cold nights (S. E. Hetherington, personal communication).

CHAPTER 5. EFFECTS OF LOW TEMPERATURE ON WHOLE PLANT PHOTOSYNTHESIS, LEAF CARBOHYDRATE CONTENT AND YIELD PARAMETERS IN DIFFERENT RICE CULTIVARS UNDER NATURAL SUNLIGHT

5. 1. INTRODUCTION

Some of the direct and indirect effects of chilling stress on photosynthesis in different rice cultivars have been clarified in previous Chapters, but further investigation is required to show how these responses affect yield parameters. In this Chapter, two aspects of rice cultivar response to low temperature treatment will be examined.

Firstly, the relationship between whole plant photosynthesis and the accumulation of carbohydrate in rice leaves during the chilling treatments simulating a CDW under natural sunlight conditions are described.

Secondly, because rice cultivars respond differently to low temperature treatment, as has been outlined in the previous Chapter, it is of interest to determine if these differences are reflected in differences in yield. I shall analyse the yield parameters affected by chilling. In this way I hope to answer the question as to whether differences in the components of yield are correlated with physiological parameters, and low temperature effects on photosynthesis.

5. 2. MATERIALS AND METHODS

Three cultivars used in the experiments described in this Chapter were cv. Hungarian-1, cv. Gui Chao-2, and cv. IR-8. The cultivation methods used in these experiments were similar to those described in the Section 2. 3, with the exception that each plant was grown in a much larger pot (15.5 cm diameter 60 cm height,) filled with soil. One litrer of nutrient solution (culture solution) was given to each pot weekly until low temperature treatment commenced. Plants were watered twice daily with tap water to maintain that nutrient solution level. They were grown in a glasshouse, 30 °C day/22-25 °C night, with natural daylight (midday PFD 1600 μmol photons m^{-2} s^{-1}), and 70% humidity.

The simulated CDW treatments were conducted in a glasshouse equipped with refrigeration unit. Temperature during the cold treatment was 17/12 °C (day/night). Maximum midday PFD was 1600 μmol photons m^{-2} s^{-1} on bright days and about 200-400 μmol photons m^{-2} s^{-1} on dull and cloudy days in the both control and chilling treatments. The humidity was uncontrolled, usually between 60 to 85%. The CO_2 concentration in these glasshouses was maintained at about 340 ± 20 μbar with an infrared CO_2 controller.

Plants were grown in the control glasshouse and then moved into the simulated CDW conditions glasshouse for a four day treatment period, when they had reached heading/flowering stages (i.e. about 50 % of tillers reached anthesis). The chilling treatment and the measurement of whole plant photosynthesis of cv. Hungarian-1 was carried

out under bright sunny weather, i.e. typical DCDW conditions. However, photosynthesis measurement of two chilling sensitive rice, cv. Gui Chao-2 and IR-8 was not done because of bad weather conditions, continuous cloudy day with rainfall occured when these plants had reached cold treatment stages. Consequently, cv. Gui Chao-2 and IR-8 were treated under natural low light conditions which simulated WCDW.

After low temperature treatment the plants were moved back to the control glasshouse until harvest. Samples for carbohydrate analysis were collected twice each day: before dawn (06:00 h), and at the end of light period (18:00 h), one 3.5 mm diameter leaf disc being taken from each of five leaves of same age during each sampling, with three plants. Three different ages of leaves (the flag leaf, second and third leaves) were sampled at the same time. Samples were frozen in liquid N_2 instantly and were later stored in a freezer. This sampling was maintained at regular intervals for a 25 day period from the heading/flowering through to the milky filling stages.

The yield parameters were measured and calculated after harvest. Plants were dried in an oven at 80 °C for about 48 h, and were then weighed with an electronic balance (Sartorius, model 1216MP). The number of grains was measured with a grain counter in the New South Wales Agricultural Institute, Yanco.

5. 3. EXPERIMENTS AND RESULTS

5. 3. 1. Effects of simulated DCDW on whole plant photosynthesis of cv. Hungarian-1

Measurements of canopy photosynthesis under simulated DCDW with artificial light in the Weiss chambers were described in Chapter 4. This section describes changes of photosynthesis in single whole plants of cv. Hungarian-1 under simulated DCDW with natural light. Figure 5. 1 shows that when plants were subjected to low temperature treatment, assimilation rate declined in the first two days of the treatment. There was a gradual but continuous recovery of photosynthesis from day 3 of the treatment even before temperature was increased. Assimilation rate was fully recovered three days after the end of low temperature treatment. Assimilation rate of control plants was fairly constant throughout the treatment period. However, there was no reduction of assimilation in control plants when measured for a short period (30 min.) at 17 °C, in contrast to the data shown in Figure 1. 2.

Conductance to water vapour transfer of the plants subject to low temperature treatment increased significantly during the treatment. On the fourth day of the chilling treatment, conductance of the treated plants was 10-fold of that of the control plants. There was a 12-fold increase in conductance of control plants measured for a short period at 17 °C. However, due to a much reduced leaf-to-air vapour pressure difference in the low temperature environment, the transpiration rates of plants during low temperature

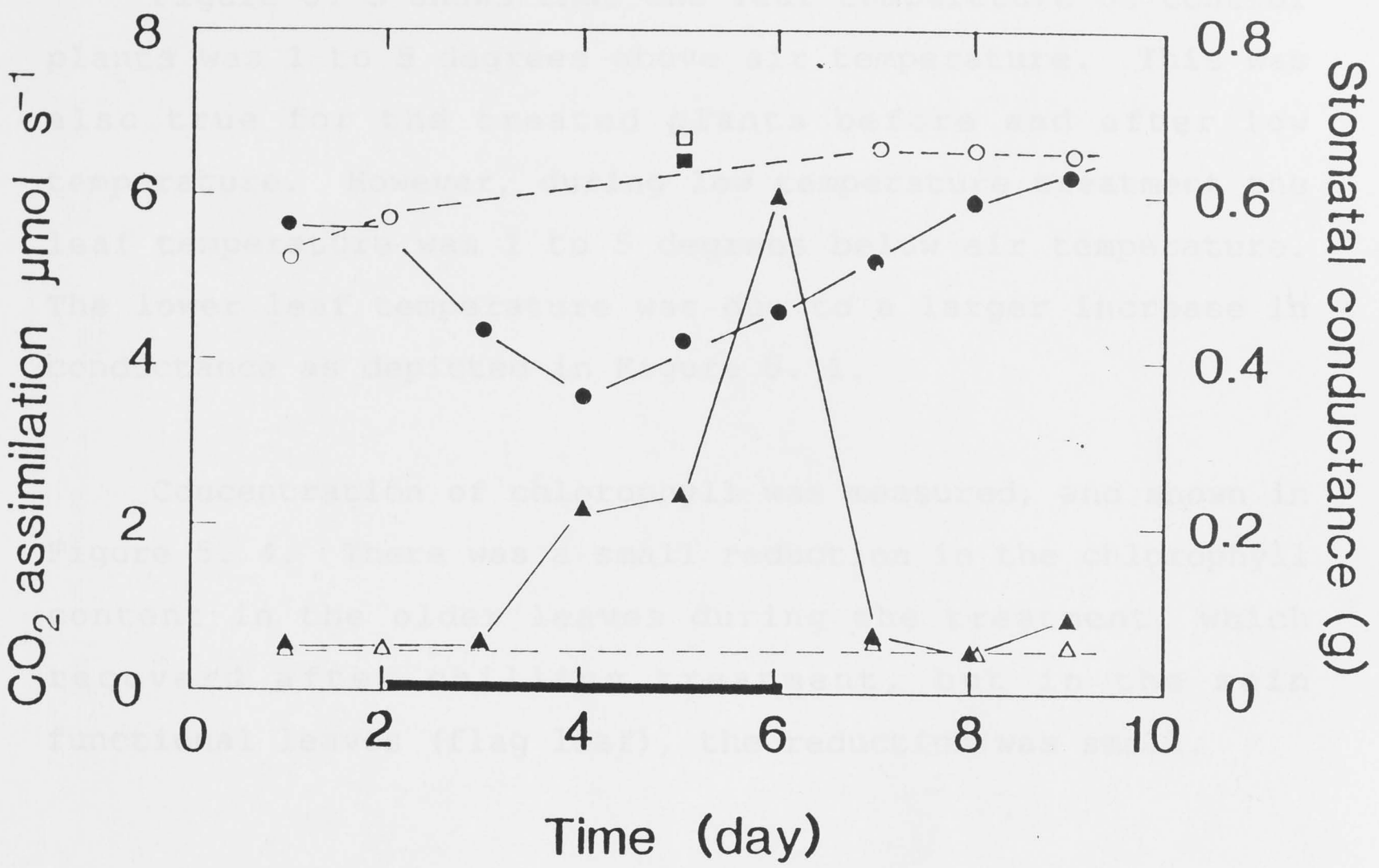

Figure 5. 1 Rate of CO_2 assimilation and leaf conductance plotted against time before, during and after low temperature treatment of cv. Hungarian-1. Symbols ● and ○ represent assimilation of treated and control plants, respectively; ▲ and Δ represent conductance of treated and control plants respectively; ■ and ❑ represent assimilation and conductance of control plants measured after exposure to chilling temperature (17 °C) for a 30 min interval. Experiments were repeated three times; only one set of data is shown.

treatment were about one-half of that of the control plants (Figure 5. 2).

Figure 5. 3 shows that the leaf temperature of control plants was 1 to 5 degrees above air temperature. This was also true for the treated plants before and after low temperature. However, during low temperature treatment the leaf temperature was 1 to 5 degrees below air temperature. The lower leaf temperature was due to a larger increase in conductance as depicted in Figure 5. 1.

Concentration of chlorophyll was measured, and shown in Figure 5. 4. There was a small reduction in the chlorophyll content in the older leaves during the treatment, which recoverd after chilling treatment, but in the main functional leaves (flag leaf), the reduction was small.

5. 3. 2. Carbohydrate accumulation in leaves of different rice cultivars under simulated CDW with natural light condition

Soluble sugars and starch content in the flag leaves of different rice cultivars were measured under simulated CDW and natural sunlight conditions. In a less chilling-sensitive rice, cv. Hungarian-1, soluble sugars content increased to a maximum level at the end of first chilling day, as observed previously (Figure 4. 8), followed by a continuous reduction of the level from second day of chilling (Figure 5. 5). The chilling-sensitive rice, cv. IR-8, showed a high level of soluble sugars in leaves which

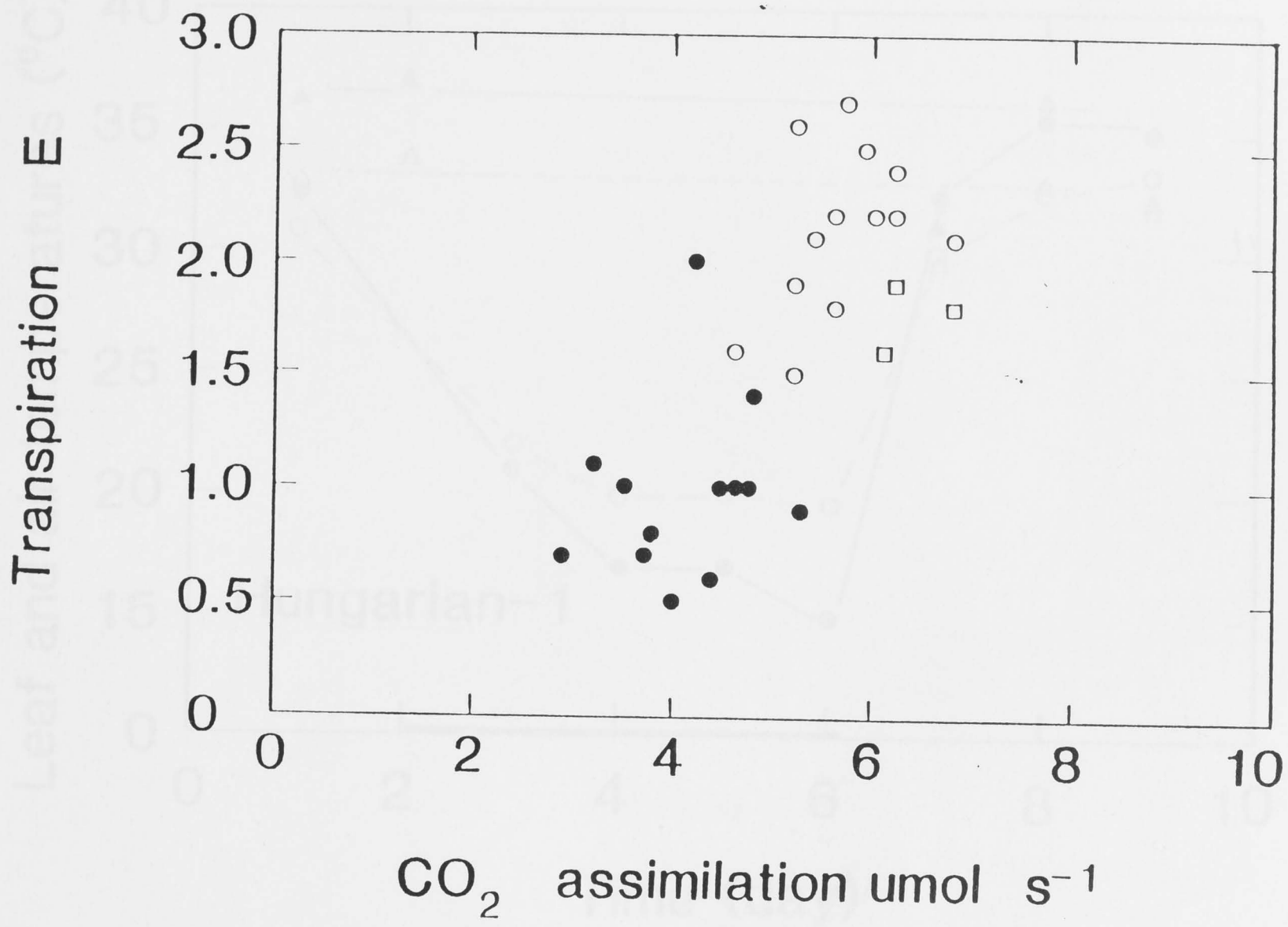

Figure 5. 2 Transpiration rate, E, plotted against rate of CO_2 assimilation, A, of control plants (○) and low temperature treated plants (●), and (□) control plants measured at chilling temperature (17 °C) for a 30 min interval. Experiments were repeated for three times, only one set of data is shown.

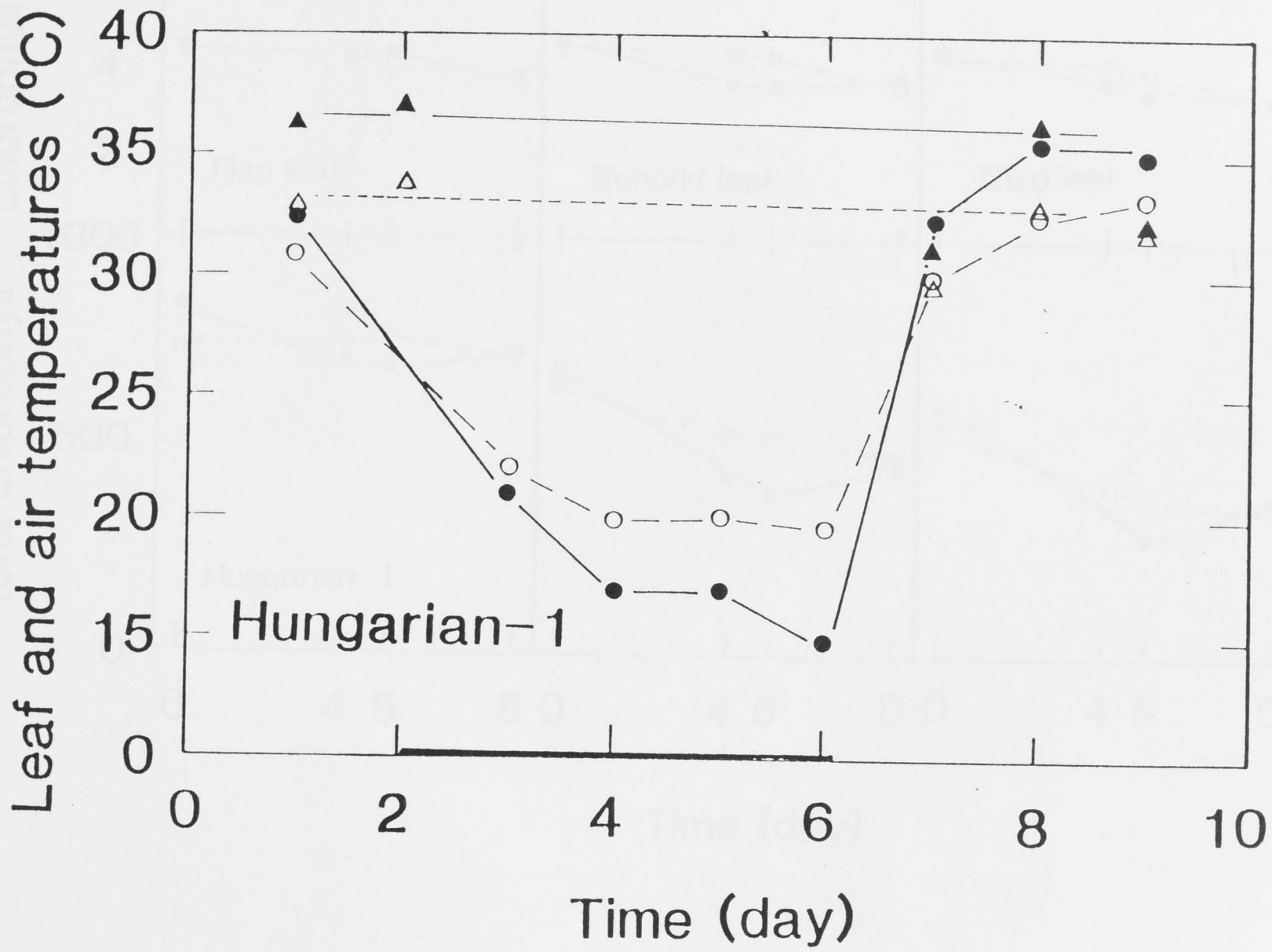

Figure 5. 3 Leaf and air temperature of control and low temperature treated plants before, during and after the treatment. Symbol ● and ○ respresent leaf and air temperature of treatment plants; ▲ and Δ represent leaf and air temperature of control plants.

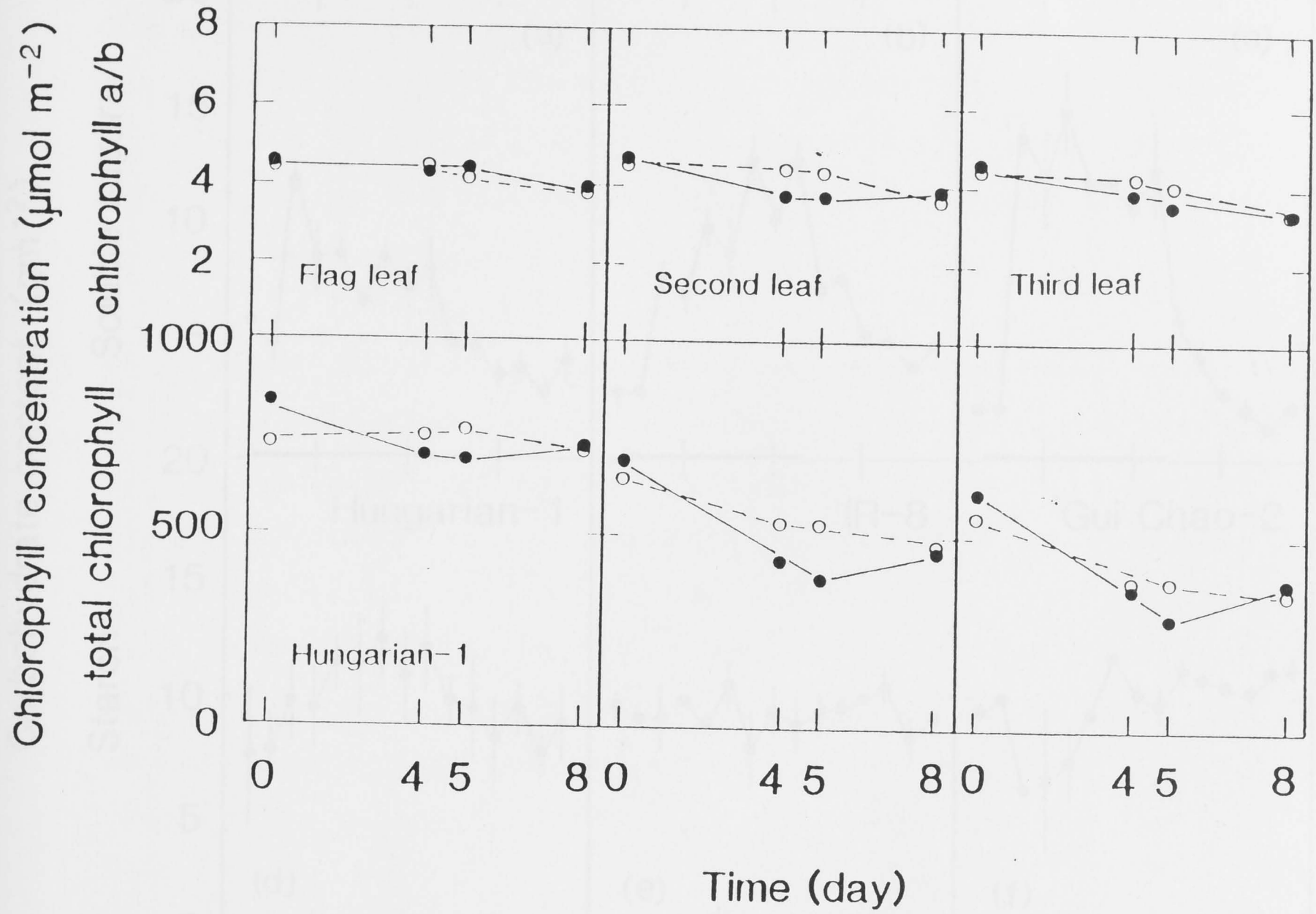

Figure 5. 4 Chlorophyll content and a/b ratio against time of flag, second and third leaves of cv. Hungarian-1. Symbols ● and ○ represent treated and control plants, respectively.

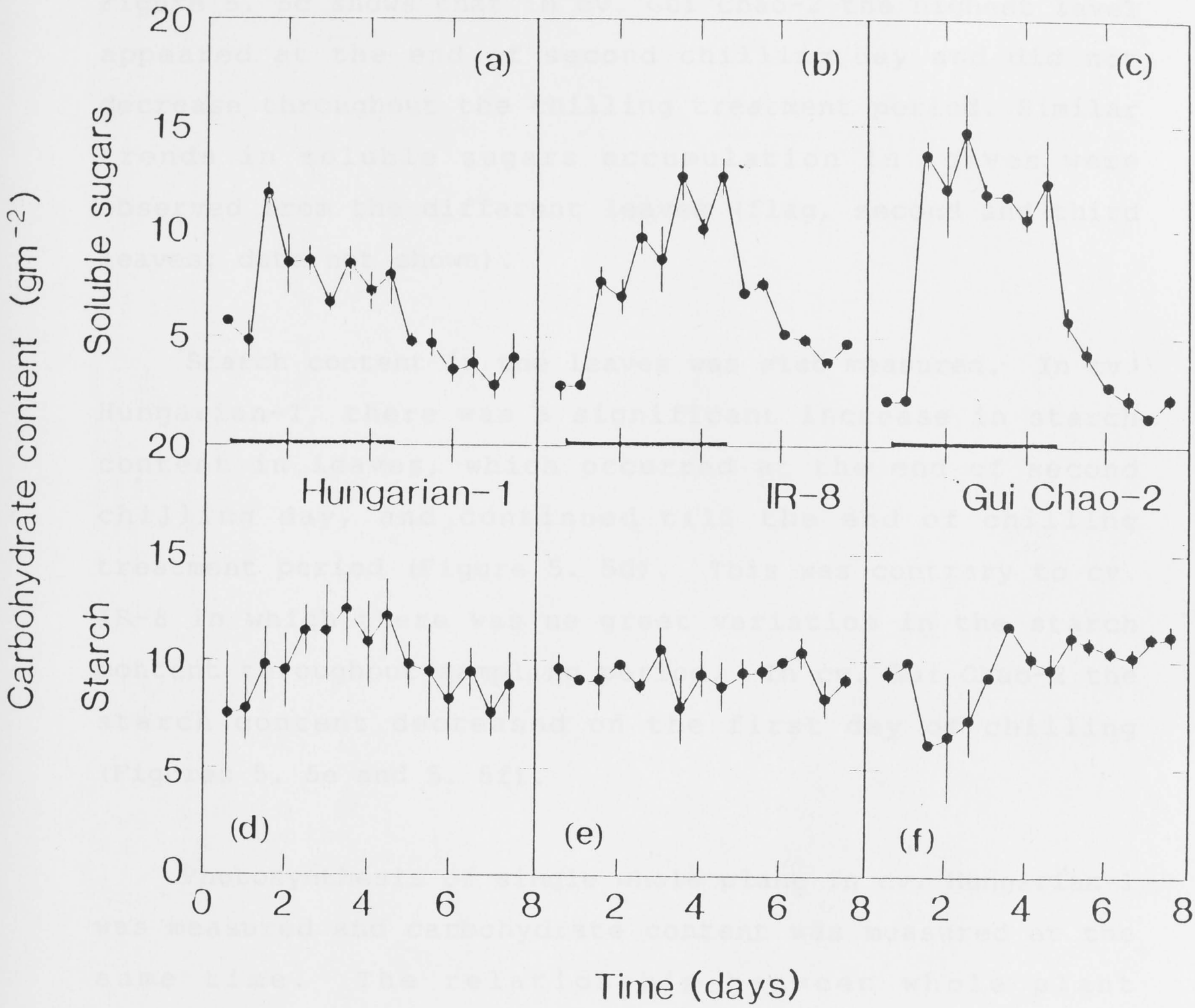

Figure 5. 5 Soluble sugars content in the flag leaf of cv. Hungarian-1 (a), cv. IR-8 (b), and cv. Gui Chao-2 (c) plotted against time. Shaded area represents the low temperature treatment interval. Starch content in the flag leaf of cv. Hungarian-1 (d), cv. IR-8 (e), and cv. Gui Chao-2 (f) plotted against time. Shaded area represents the low temperature treatment interval.

was maintained till end of chilling period (Figure 5. 5b), Figure 5. 5c shows that in cv. Gui Chao-2 the highest level appeared at the end of second chilling day and did not decrease throughout the chilling treatment period. Similar trends in soluble sugars accumulation in leaves were observed from the different leaves (flag, second and third leaves; data not shown).

Starch content in the leaves was also measured. In cv. Hungarian-1, there was a significant increase in starch content in leaves, which occurred at the end of second chilling day, and continued till the end of chilling treatment period (Figure 5. 5d). This was contrary to cv. IR-8 in which there was no great variation in the starch content throughout sampling period. In cv. Gui Chao-2 the starch content decreased on the first day of chilling (Figures 5. 5e and 5. 5f).

Photosynthesis of single whole plant in cv. Hungarian-1 was measured and carbohydrate content was measured at the same time. The relationship between whole plant assimilation rate and soluble sugars content in leaves of treated plants shows a negative correlation (Figure 5. 6). The second leaves accumulated more soluble sugars relative to the flag leaves. There was an inverse relationship between starch content in the flag and second leaves and the assimilation rates of the treated plants (Figure 5. 7).

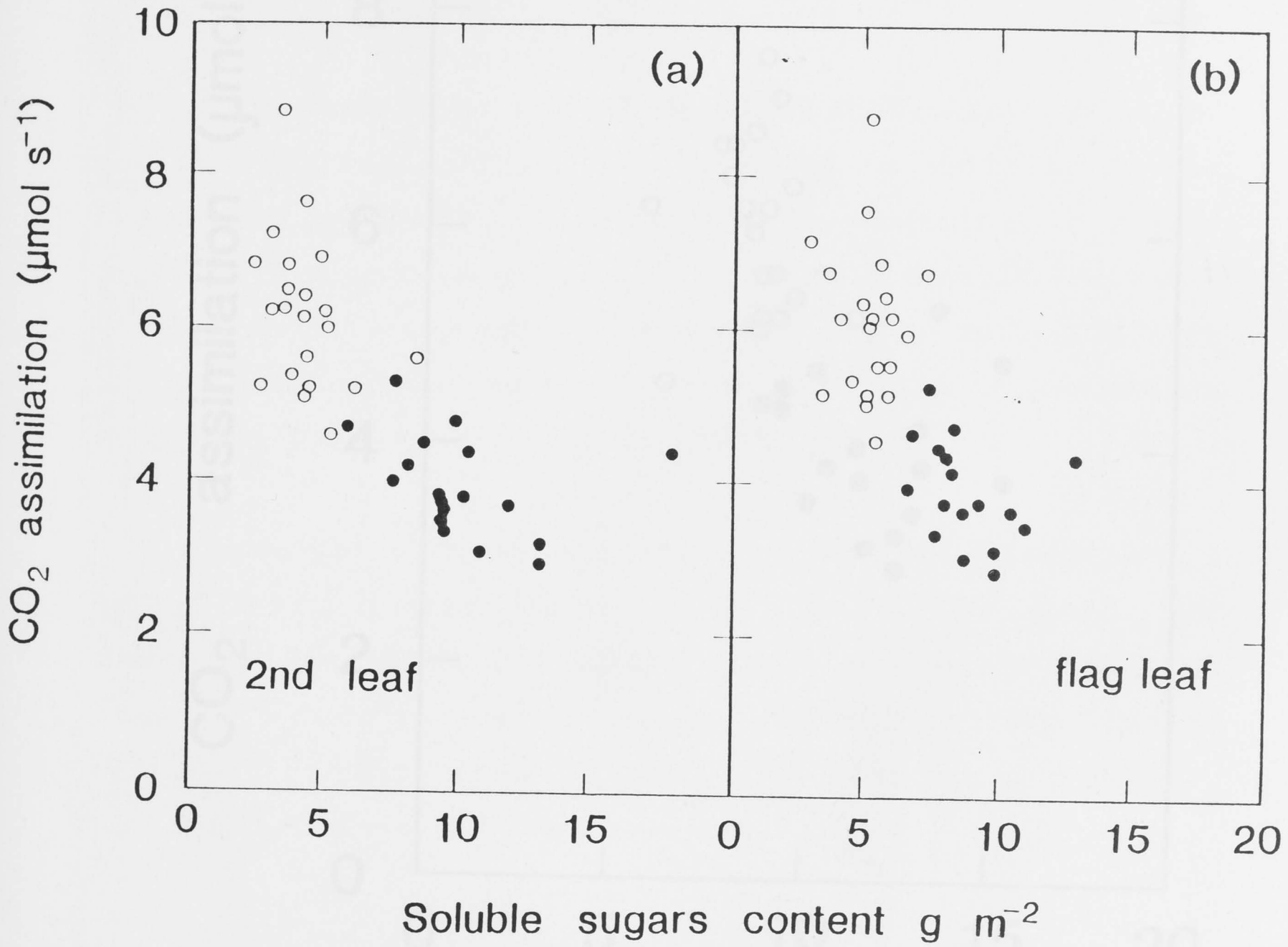

Figure 5. 6 Soluble sugars contents as a function of CO_2 assimilation rates of low temperature treatment (●) and control (○) plants (cv. Hungarian-1)

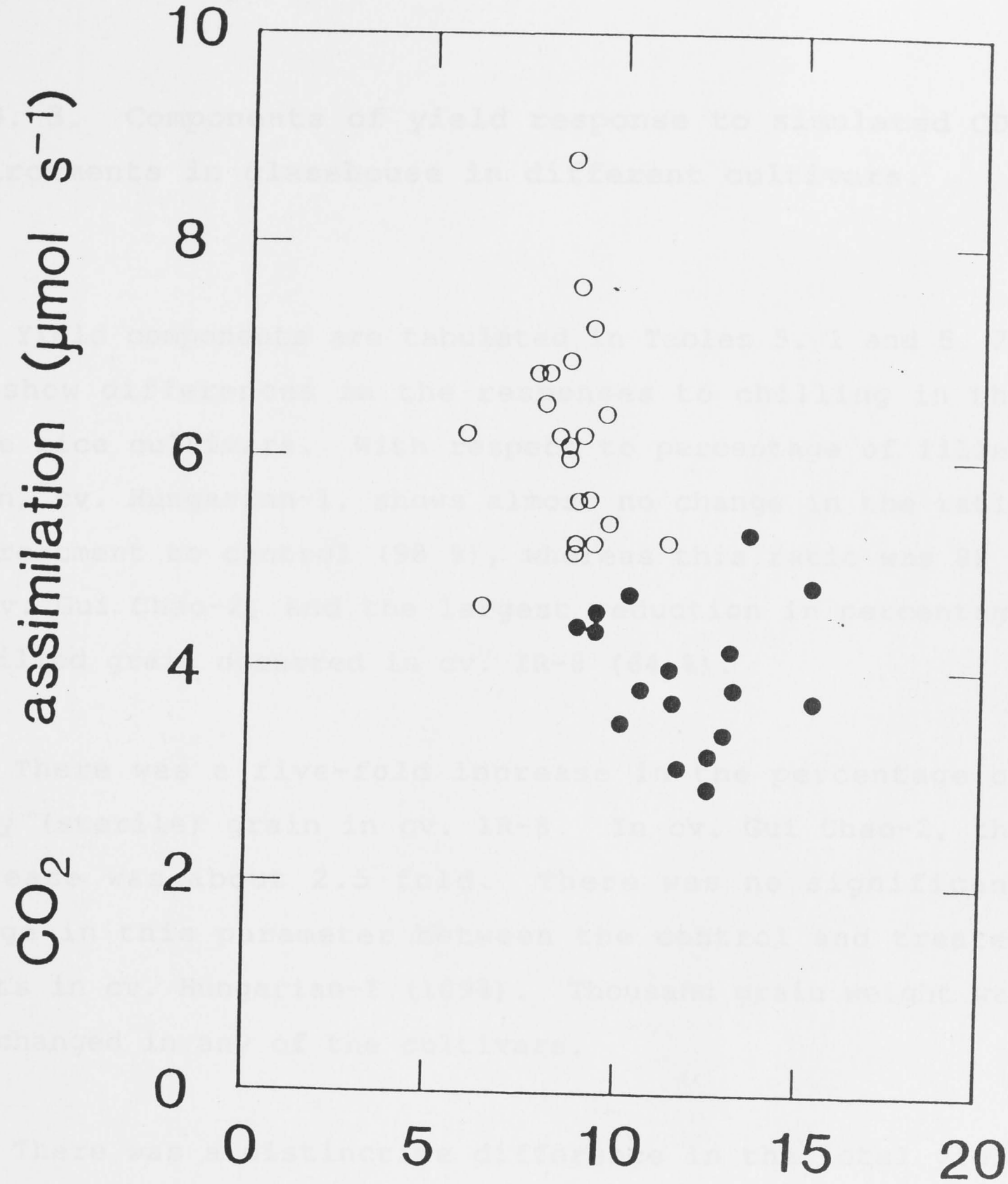

Figure 5. 7 Starch contents as a function of CO_2 assimilation rates of low temperature treatment (●) and control (○) plants (cv. Hungarian-1) (flag leaf).

5. 3. 3. Components of yield response to simulated CDW environments in glasshouse in different cultivars.

Yield components are tabulated in Tables 5. 1 and 5. 2, and show differences in the responses to chilling in the three rice cultivars. With respect to percentage of filled grain, cv. Hungarian-1, shows almost no change in the ratio of treatment to control (98 %), whereas this ratio was 88 % in cv. Gui Chao-2, and the largest reduction in percentage of filled grain occurred in cv. IR-8 (64 %).

There was a five-fold increase in the percentage of empty (sterile) grain in cv. IR-8. In cv. Gui Chao-2, the increase was about 2.5 fold. There was no significant change in this parameter between the control and treated plants in cv. Hungarian-1 (109%). Thousand grain weight was not changed in any of the cultivars.

There was a distinctive difference in the total yield between chilling sensitive and insensitive rice cultivars after 4 days simulated CDW treatment. Hungarian-1 shows a reduction of only 9.7% of filled grain weight, compared with a 13.2% reduction in Gui Chao-2, and 36.8% reduction in cv. IR-8.

The ratio of filled grain weight to stem weight, is an indication of photosynthate distribution, and translocation. The three cultivars showed differences in the responses of

Table 5. 1 Components of yield response to simulated CDW environments in the glasshouse with three cultivars of rice (Percentage change compared to control shown in parenthesis)

Yield component	Hungarian-1		Gui Chao-2		IR-8	
	Control	treatment	control	treatment	control	treatment
Filled Grains (%)	86.8±2.4	85.3±1.9 (98%)	73.6±3.2	64.8±4.7 (88%)	81.8±5.8	52.3±4.7 (64%)
Half Filled Grains (%)	5.1±0.5	5.3±0.4 (104%)	14.6±1.2	5.0±3.9 (34%)	8.1±2.5	0.9±3.3 (11%)
Empty grains (%)	8.5±1.8	9.3±1.6 (109%)	11.8±2.0	30.2±7.9 (256%)	10.1±3.3	46.8±7.6 (463%)
Filled grain weight (per plant)	73.4±3.2	66.3±3.2 (90.3%)	87.1±4.0	75.6±11 (86.8%)	77.8±6.2	49.2±7.5 (63.2%)
Filled grain/stem (weight)	1.63±0.02	1.54±0.02 (94.5%)	1.22±0.02	1.03±0.14 (84.4%)	1.10±0.05	0.60±0.10 (54.6%)
1000 grains weight (1)	32.4±0.2	31.5±0.4 (97%)	25.3±0.4	26.2±0.9 (104%)	26.3±0.9	25.5±3.4 (97%)
Filled grains per plant (2)	1983±74	1817±90 (92%)	3444±112	2906±523 (84%)	2968±276	1943±298 (65%)

Table 5. 2 Components of yield response to simulated CDW environments in the glasshouse with three cultivars of rice (Percentage change compared to control shown in parenthesis)

Yield component	Hungarian-1		Gui Chao-2		IR-8	
	Control	treatment	control	treatment	control	treatment
Plant height (cm)	/	/	92.0±3.9	80.1±0.2	75.4±1.8	65.2±1.5
		/		(87%)		(87%)
Panicle length (cm)	21.6±2.1	20.6±2.5	21.4±0.4	20.6±0.9	22.4±0.3	21.6±0.3
		(95%)		(96%)		(96%)
Neck binding of panicle	0	0	-1.2±0.1	-6.3±2.1	-1.6±0.8	-5.8±0.6
(cm)		0		(525%)		(363%)
Number of panicles	18.8±1.0	18.0±1.2	24.9±2.6	25.3±3.2	26.9±2.4	27.8±2.4
		(96%)		(102%)		(103%)
Number of filled grain	105.9±5.4	101.6±5.2	152.3±8.2	126.1±20.0	114.6±12.4	68.6±11.4
per panicle		(96%)		(83%)		(60%)
Number of spikelets	122.7±2.4	119.0±1.9	207.2±8.7	193.4±18.9	140.6±16.2	130.4±12.0
per panicle		(97%)		(93%)		(93%)
Stem weight per plant	39.4±1.3	38.6±1.9	71.4±3.8	73.7±3.2	70.9±5.4	82.4±4.4
		(98%)		(103%)		(116%)

this parameter to chilling. Calculation from data in Table 5. 1 shows that in cv. Hungarian-1 there was a reduction of 5.5%, compared to 15.6% reduction in Gui Chao-2, and 45.4% in IR-8. This comparison suggests that effects of simulated CDW on translocation of photosynthate is much greater in chilling sensitive cultivars than in chilling tolerant cultivars. However, we must remember that the low light conditions experienced by cv. Gui Chao-2 and IR-8 could have exaggrated the difference.

5. 4. DISCUSSION

Whole plant photosynthesis measurements reported in this Chapter confirm the canopy data given in Chapter 4. In cv. Hungarian-1 assimilation rate was reduced during the first two days of low temperature treatment, and then began to recover, being fully restored three days after chilling treatment ceased. The inhibition was greater than in Figure 4. 8, possibly because the leaf temperatures were significantly lower than in the chamber experiments. Chlorophyll content of leaves did not change during the treatment (Figure 5. 4), further supporting the notion that direct effects of chilling on the primary photochemical process and photoinhibition do not account for the decrease in photosynthesis and grain yield.

These results help us to decide if effects of low temperature on the photosynthesis are due to (1) decreased stomatal conductance (increased stomatal resistance) which

leads to reduced CO_2 exchange; (2) increased photosynthate accumulation in the leaves which leads to a feedback inhibition of photosynthesis; or (3) changes in the transpiration rate of treated plants which lead to water stress and limitation in translocation of photosynthate from leaves to other organs.

However, in cv. Hungarian-1 conductance to water vapour transfer increased significantly, up to about 10-fold at day four of chilling treatment and was followed by reduction of assimilation. This is unlinely to cause water stress during the simulated DCDW environments (due to low leaf-to-air vapour pressure difference) and unlikely to impede CO_2 uptake in photosynthesis. On the other hand, Figures 5. 5a and 5. 5d show that an increase in soluble sugars and starch coincided with a reduction of the assimilation rate, and as soluble sugars content declined after the treatment, assimilation rate increased. This suggests that this reduction of photosynthesis was due to increase in soluble sugars content in leaves. As emphasised above, cv. Hungarian-1 shows larger responses in these experiments, compared to those in Chapter 4, because lower day temperatures were used.

As a limiting factor temperature seems to have two effects, short term and long term. In other words, the type of interaction between photosynthesis and temperature may be time-dependent. For example, on the longer time scale, low temperature caused a 35% reduction in CO_2 assimilation rate two days after the imposition of chilling treatment (see Figure 5. 1). However, on a shorter time scale (30 min.),

there was no change when control plants was measured at chilling treatment temperatures.

In contrast, both short and long time of treatment had similar effects on stomatal conductance in cv. Hungarian-1, causing it to increase by about 10-12 fold. This is similar to the reports of Crookstoon *et al.* (1971); Drake and Salisbary (1972); Neilson *et al.* (1972); Neilson and Jarvis, (1975), but is contrary to the results obtained from cv. HKHY, EZZ, HQ, and WZ-3 in Guangzhou (Table 4. 5). I do not know the reason for the differences in the stomatal response to chilling treatment. It could be that different cultivars responded differently. In the case of cv. Hungarian-1 grown in the glasshouse, a 12-fold increase in conductance seems alarming, but the low leaf temperatures during chilling treatment gives further evidence to the larger increase in conductance (Figure 5. 3). However, due to the small leaf-to-air vapour pressure difference (about 2 mbar) during chilling treatment, the actual transpiration rate was about one-half of the control plants (see Figure 5. 2). Without leaf water potential measurements, it would be difficult to say whether the chilling treatment also subjected the plants to water stress. This would be dependent on the degree of reduction of hydraulic conductance in the chilled roots.

The data given here confirm that different patterns of soluble sugars and starch accumulation were found in different chilling sensitive cultivars exposed to low temperature under natural sunlight. In chilling tolerant rice, cv. Hungarian-1 (Figure 5) the maximum content of soluble sugars accumulated in leaves was observed at the end of first chilling day, and then decreased throughout the

simulated CDW treatment period. Starch content began to increase in the first chilling day, and continued to the end of the treatment. Different results were observed from chilling sensitive rice, cv. Gui Chao-2 and IR-8. Maximum content of soluble sugars was observed in the end of second chilling day in cv. Gui Chao-2, but this high sugar content was stable during the chilling period. Lower starch content in first chilling day, was then followed by an increase during the treatment period was in this cultivar (Figure 5). Increase of content of soluble sugars was maintained, with no change in starch content, in cv. IR-8 during the simulated CDW period.

The high content of soluble sugars accumulated in leaves of all cultivars at the end of first chilling day perhaps serves to increase the osmotic pressure of leaf cells, thus offsetting the possibility of water stress which might have occurred, associated with high conductance. The accumulation of sugars may reflect reduced translocation of photosynthates, as well as hydrolysis of starch. From these results, I suggest that, in the longer term:

(1) chilling resistant rice cultivars, such as cv. Hungarian-1, have adapted to low temperature environments, and can progressively transform soluble sugars to starch and store it in leaves, thus avoiding feedback inhibition.

(2) in chilling sensitive rice cultivars, such as cv. Gui Chao-2, the starch content is reduced in the first chilling day, contributing solutes for osmotic purposes, as well as an increase in the concentration of soluble sugars for adaptation to water stress which may be associated with

low temperature. However, this has inhibitory effects on photosynthesis.

(3) In the other chilling sensitive rice, cv. IR-8, starch content was maintained on a stable level throughout, and concentration of soluble sugars gradually increased during chilling. This seems to be intermediate between the two extreme responses to chilling stress developed above.

Park and Tsunoda (1983) showed that the chilling stress (17/12 °C, day/night, with natural light conditions) led to increase in the carbohydrate accumulation in leaves of rice seedling stage. They considered that at first soluble sugars increased, then starch increased. Varietal differences were also found in the speed of carbohydrate accumulation, which led to reduced leaf photosynthetic rate during these chilling events. As discussed in Chapter 4, mechanisms of these interactions are not well known, and the cultivar differences described by Park and Tsunoda (1983), and in my experiments, need to be explored further. The variation in patterns of carbohydrate metabolism in response to low temperature are significant parameters which might indicate the extent of changes in the activity of enzymes *in-vivo* in rice leaves during chilling stress.

The question of whether effects of low temperature (simulated CDW) during grain filling have an effect on photosynthesis which is sufficient to reduce yield, needs to be considered. As discussed in Chapter 1, chilling effects on sterility are believed to explain most effects of low temperature on rice yield. My attempts to assess the relative importance of sterility against reduced

photosynthate production or translocation are incomplete. However, the analysis of yield components provides some understanding (Tables 5. 1 and 5. 2). The greater reduction in total grain weight in cv. IR-8 and cv. Gui Chao-2 was clearly associated with greater sterility (more empty grains) following chilling. In spite of the care taken, such a response could arise from small differences in timing and duration of floral development in relation to the chilling treatment in these cultivars compared with cv. Hungarian-1. There is no doubt that sterility was the major contributor to overall yield reduction in these experiments. However, two other yield parameters indicate that photosynthate production and/or translocation, also played a role in yield reduction in cv. IR-8 and cv Gui Chao-2. These are, first, a large decrease in the percentage of half filled grains, and second, a large decrease in the ratio of filled grain weight to stem weight.

There was very little effect of chilling on these yield components in cv. Hungarian-1. Evidently the absence of effects of chilling on sterility in Hungarian-1 correlate well with its insensitivity to chilling as judged by fluorescence parameters, photosynthesis, carbohydrate changes and general phenology. However, we should also consider that effects of low temperature on yield due to reduced photosynthate supply and translocation during the chilling treatment may be compensated by rapid recovery of photosynthesis after chilling, provided chilling has not impaired panicle development. Such may be the case in cv. Hungarian-1. It could be argued that this compensation is even more likely in chilling sensitive cultivars in which sterility has already reduced the number of grains to be

filled. In this case, reduction in half-filled grains and in ratio of filled grain weight to stem weight suggest even greater effects of low temperature on photosynthesis and yield in cv. IR-8 and cv. Gui Chao-2.

Thus the percentage of half filled grain may indicate relative chilling tolerance in rice cultivars due to effects on photosynthesis. This parameter may also be an indicator for the effects of low temperature on photosynthate translocation from leaf to panicle.

Zhang *et al.* (1984b) found that mean of daily temperature 16 °C did not reduce 1000 filled grain weight. They explained that this might be due to a "recovery effect", because plants enjoyed a long period for recovery after simulated CDW events in their experiments. They also suggested that 1000 filled grain weight may depend on the availability of photosynthate under stress environments. No differences in this yield parameter were found in chilling sensitive or tolerant cultivars after simulated CDW conditions in my experiments.

CHAPTER 6. GENERAL DISCUSSION

The research described in this thesis has been concerned with the physiological basis of how low temperature, particularly Cold Dew Wind (CDW) conditions, affect photosynthesis and the yield of rice plants, especially during the late stages of the second rice crop in South China. Three main questions were posed: 1. Do leaves of rice cultivars differ in their sensitivity to interaction between high light and low temperature? This was measured using chlorophyll fluorescence and photosynthetic quantum yield techniques with a view to applying these methods for screening chilling tolerant cultivars in a breeding programs. 2. Do above processes indicate damage to primary processes of photosynthesis under simulated CDW events in controlled environments which account for reduced carbon assimilation? 3. If they do not, are other physiological processes correlated with depressed photosynthesis under these conditions, and can these be used to screen chilling tolerant cultivars? Do these processes account for reduced impaired carbon assimilation, and is the reduced carbon assimilation responsible for reduced yield?

Initially, the research aims revolved around the need to develop rapid methods for screening chilling tolerant rice cultivars, using chlorophyll fluorescence. Chlorophyll fluorescence methods for screening chilling tolerant plants were developed by Hetherington and Smillie (1983, 1984) and validated by comparative measurements on a wide range of plants known to differ in chilling tolerance. Over 100 different species and 1000 different cultivars had been ranked for chilling tolerance.

However, compared with other plants species, it was found that rice leaves were not very sensitive to low temperature (Smillie *et al.*, 1988). Terashima *et al.* (1989) showed that exposure to 0 °C in the dark for 48 h, which causes dissociation of extrinsic thylakoid proteins of the PS II water splitting complex in chilling sensitive cucumber, had no effect in rice. Exposure to 5 °C, 500 μmol photons m^{-2} s^{-1} for 5 h, which causes dissociation of CF_1 subunits CF_o of the thylakoid ATPase in cucumber, had only little effect in cv. IR-8.

As outlined and described in Chapter 2, my results confirm that rice plants may naturally experience a coincidence of bright light and low temperature in the early daylight hours during Cold Dew Winds. Chilling temperature exaggerates photoinhibition (Chapter 3), but chilling in the dark did not increase sensitivity to photoinhibition under bright light at room temperature when chilled plants (at 10 °C in the dark for 12 h) were exposed to 700 μmol photons m^{-2} s^{-1}, 25 °C for 8 hours. When detached leaves were exposed to simulated CDW environments, changes in 77K fluorescence and quantum yield were observed. However, the extent of decrease in F_v/F_m and increase in F_o seemed to relate more to leaf orientation than to any effect on rate of photosynthesis in air during the low temperature and bright light treatment (see Chapter 4). As described in Chapter 4, simulated DCDW experiments suggest that changes in primary photochemical reactions, as indicated by quantum yield of photosynthesis and chlorophyll fluorescence were not associated with significant changes in photosynthetic CO_2 assimilation. In the field most leaves of the canopy are under shade conditions and the natural orientation of the leaves is such that they experience about 300 μmol m^{-2} s^{-1} of PFD under maximum sunlight. This implies that

photoinhibition is unlikely to occur. It seems that photochemical reactions of photosynthesis are not responsible for reduction of photosynthesis in rice plants under the simulated DCDW treatment event or in natural paddy field conditions. Some field experiments further support this view (see Chapter 4.). Therefore the rapid methods for screening chilling tolerant cultivars in rice plants using chlorophyll fluorescence may need further investigation to substantiate its usefulness.

Consequently, the research interest was shifted from the photochemical reactions to other physiological responses of rice to low temperature. I found that effects of low temperature on photosynthesis were first due to the accumulation of photosynthate in the leaves. There was a good correlation between soluble sugars accumulation in the leaves and reduction in the photosynthesis both of single plants and the canopy. In Chapters 4 and 5 these physiological interactions have been discussed.

Previous studies in this field (Sato and Park, 1981) attempted to link carbohydrate metabolism to changes in light reactions, via effects on chloroplast membranes following starch accumulation. Low temperature (15-10℃) increased the number and volume of starch grains accumulating in leaves of rice seedlings. Sato and Park (1982) and He (1985) reported that chilling (14 ℃ for 3 days) hybrid rice, cv. Shan You-6 did not damage the thylakoid membranes, but increased the numbers and volume of starch grains in chloroplast which in turn distorted the structure of thylakoid and caused chloroplast swelling. Similar results from the different types of rice cultivars, such as the cold-sensitive Indica rice, Indica x Japonica rice and the cold-resistant Japonica rice cultivars were

shown by Park *et al.* (1979). They also found that the size of chloroplasts increased after low night temperatures, as indicated by larger packing volume. They suggested that the translocation of photosynthate from chloroplasts might be impeded and consequently interfere with photosynthesis. My conclusions are different in that I think soluble sugars content in leaves is the most important factor in chilling stress effect on photosynthesis, because the accumulation of soluble sugars seems to have a feedback inhibition on photosynthesis.

As discussed above, the project aim was to devise a method for selecting chilling tolerant plant cultivars. The content of soluble sugars in the leaf in rice plants is a most sensitive parameter when plants are exposed to low temperature environments. A screening method should be developed using soluble sugars as an indicator to test the resistance to low temperature. However, further research in this field, identifying the relative concentration of each sugars at different degree of chilling stress, is required.

More research is needed to separate the relative effects of low temperature on sterility and on photosynthate supply as factors responsible for yield reduction under the particular conditions which apply in South China. My experiments were incomplete, for reasons beyond my control, but there were clear indications that low temperature effects on photosynthate production and transport contributed to yield reduction in cv. IR-8 and cv. Gui Chao-2. This was in addition to the large effect of low temperature on sterility, especially in cv. IR-8. These data suggest an interesting avenue to study causes and effects of low temperature environment on yield which may be

linked to screening procedures based on sugar analyses and applied directly to breeding programs.

At the end of this thesis I hope that my research has made some significant contribution in rice chilling research, and can be useful to rice breeding programs in the search for chilling tolerant rice cultivars, which in turn will improve rice production. I also hope that the report may be a new starting point in the study field of chilling stress in rice plants!

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